MARSHALL'S PHYSIOLOGY OF REPRODUCTION

FOURTH EDITION

VOLUME 3

PREGNANCY AND LACTATION

PART ONE

OVULATION AND EARLY PREGNANCY

EDITED BY G.E. LAMMING

SPRINGER-SCIENCE+BUSINESS MEDIA, B.V.

MARSHALL'S PHYSIOLOGY OF REPRODUCTION

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Fourth edition

Volume 3 Pregnancy and Lactation

Part One
Ovulation and early pregnancy

Edited by

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PREFACE

In 1910 F.H.A. Marshall established a landmark in the history of science when he published the first edition of *The Physiology of Reproduction*. A revised second edition was published by Marshall in 1927 and in this he was assisted by a young graduate, A.S. Parkes (later Sir Alan), who accepted an invitation to organize and edit the third edition, the preparation of which was planned in 1939 and then delayed by the advent of the Second World War. It was subsequently completed in three volumes, the first of which appeared in 1956 and the final volume in 1966.

For nearly a century succeeding editions of *Marshall's Physiology of Reproduction* have provided an advanced reference text for reproductive biologists. We hope the current volume will maintain this role.

Organizing and editing the work of the Fourth Edition has involved an extension of the scope of each succeeding text while retaining a comprehensive philosophical approach to the review of literature. The coverage demanded for such an advanced text has expanded exponentially during the last decade.

For the Fourth Edition, Volume 1 *The Reproductive Cycles of Vertebrates* published in 1984, provided a synopsis of the various reproductive patterns. It contains major contributions concerning fishes, reptiles, amphibia, birds, marsupials and eutherian mammals. The volume included special chapters on Non-human Primates and Humans.

Volume 2 on *Reproduction in the Male*, published in 1990, provided a detailed analysis of research on male reproductive physiology, male life cycles and seasonality, the physiology of the testis and accessory sexual organs, artificial insemination and the preservation of semen.

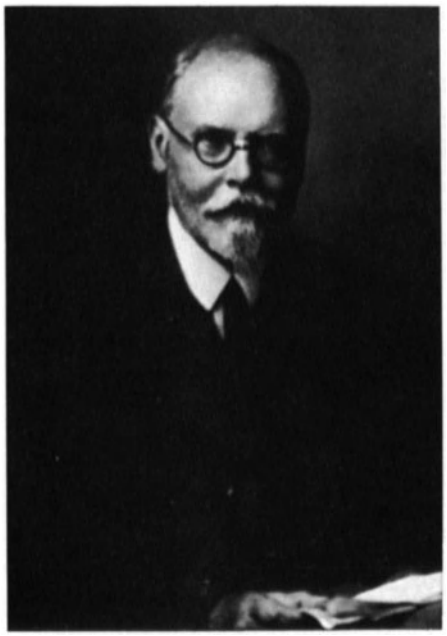
The current volume aims to maintain Marshall's traditional comparative approach with particular emphasis on reviewing research data derived from studies of farm and laboratory animal species, non-human primates and humans. A major effort has been made by contributors in the final revision of their texts to present the results of the most recent research. However, given the time required to receive and edit the manuscripts, check the proofs and index the volume, it is as fully up-to-date as possible. The increasing impact of new information on the reproductive physiology of humans is well reviewed and this aspect amplifies the valuable interrelationship which exists between those working experimentally with farm and laboratory species and those engaged in the control of reproduction and assisted conception in humans. The comparative aspects of placentation, embryo and fetal development, the initiation of parturition and lactation are extensively reviewed.

It is our established editorial policy to seek contributions from the most eminent international authorities. This I trust we have achieved in full measure. I acknowledge and welcome the contributions made by the authors from Australia, New Zealand, Israel, the United States of America, as well as those from the UK.

G.E. Lamming
University of Nottingham

A BIOGRAPHICAL NOTE PREPARED BY *SIR ALAN PARKES*

(for the Third Edition)



F. H. A. MARSHALL
CBE, MA, PhD, DSc, ScD, FRS.
11th July 1878–5th February 1949

Marshall's name is associated, mainly or even exclusively, with the study of the physiology of reproduction in its many branches. He was preceded in this field by another pioneer, Walter Heape, who was completing his classic studies of the primate uterus and collecting material for his analysis of the oestrous cycle at the time when Marshall was an undergraduate in Cambridge. The two men did not meet until later, but they corresponded freely, and Heape's morphological studies and extensive knowledge of living animals were of great value to Marshall in his early days. Heape, however, was not a physiologist – he seems, for instance, to have regarded the ovary merely as a producer of eggs – and it is to Marshall that we owe the foundations of our present knowledge of the role of internal secretions in the reproductive processes.

Marshall's debut was characteristic of an age in which research was a calling rather than a profession; a precarious calling to which young men were drawn by interest, not by ambition. In 1900, the late Lord Carmichael gave £200 to Prof. Cossar Ewart, Professor of Natural History in the University of Edinburgh, to enable a Research Student to assist with his long-term experiments on telegony, and to undertake work on reproduction in farm animals. Cossar Ewart's inquiries resulted in Marshall accepting an invitation to go to Edinburgh. The work on telegony, which involved a detailed study of the hair of the Equidae, was uninteresting and unproductive, and

Marshall turned his attention to research on the sexual cycle in the sheep, for which he was to make use of Ewart's farm at Penicuik. This work was the subject of his first important scientific publication, which appeared in the *Transactions of the Royal Society* in 1903. Similar studies followed with Schäfer on the oestrous cycle in the ferret (1904), and with Jolly on the oestrous cycle in the dog (1905). A communication on the ovary as an organ of internal secretion, written in collaboration with Jolly and published in the *Transactions of the Royal Society* in 1905, constituted the first serious attempt to correlate the changes in the uterus during the reproductive cycle with the cyclic production of different internal secretions by the ovary.

Marshall returned to Cambridge in 1908, and continued his work on the physiology of reproduction. During the war, 1914–18, he studied, on behalf of the Ministries of Food and Agriculture, such problems as the most economic age and condition at which to slaughter cattle. Subsequently, Marshall's researches have dealt mainly with the influence of exteroceptive factors – light, climate, etc. – on the reproductive processes, and in 1936 he made this the subject of his Croonian Lecture. He was also intensely interested in the courtship and breeding behaviour of birds, on which he was a considerable authority.

Marshall spent a great deal of time preparing the first edition of *The Physiology of Reproduction* in which he gathered together all relevant knowledge concerning the breeding season, cyclic changes in the reproductive organs, fertilization, fertility, lactation and so on. The book was published by Longmans, Green & Co. in 1910, and immediately achieved success. A second edition was called for after the 1914–18 war. This second edition, published in 1922, was necessarily much larger than the first, for since 1910 knowledge of the reproductive processes in mammals and lower vertebrates had increased considerably. A third edition, largely rewritten by Marshall's pupils and colleagues, and again much enlarged, is now in the course of publication.

Marshall's main contribution to biology has been twofold. In the first place, the stimulating synthesis of existing knowledge embodied in the first edition of *The Physiology of Reproduction* gave impetus and balance to a branch of biology sadly lacking in both. The fact that between the wars the physiology of reproduction became a subject of intensive study by scientists in all countries was due in no small measure to the stimulus given by Marshall's writings. In the second place, Marshall's remarkable prescience in deducing correctly the existence and respective roles of follicular and luteal hormones long before either was isolated, or even obtained in active extracts, had a profound influence on the development of the subject. During the early years of the century Ludwig Fraenkel in Breslau and P. Bouin in Strasbourg, working on the rabbit, concentrated attention on the corpus luteum; in the 1920s intensive study of the follicular hormone by Edgar Allen and his colleagues in the USA swung attention to the follicular apparatus. It fell to the English school of workers, under Marshall's influence, to hold the balance until the final demonstration and isolation of the corpus luteum hormone in G.W. Corner's Rochester laboratory completed the working out of his early predictions.

Scientists are of many kinds, but inspiration flows most fruitfully from those who are able, by some gift withheld from lesser men, to divine the richness of uncharted country and sense the vital landmarks. Thus do they avoid the barren places and the morasses of unimportant detail which engulf so many. To these, discovery is an art rather than a science, a matter of instinct rather than of intellectual machinery. Such was Marshall.

20 October, 1948 A.S.P.
Revised 10 February, 1949.

F.H.A. MARSHALL

CURRICULUM VITAE

Francis Hugh Adam Marshall was the younger son of the late Thomas Marshall, J.P., and was born at High Wycombe on 11 July, 1878. He was educated at St. Mark's School, Windsor, and privately. After a short time at University College, London, he went to Christ's College, Cambridge, in 1896, and took the Natural Science Tripos in 1899. During the latter part of the time in Edinburgh Marshall became Carnegie Fellow and Lecturer on the physiology of reproduction. After his return to Cambridge he was Lecturer and then Reader in Physiology, in the School of Agriculture, for more than 35 years, until 1943. For a short period, Marshall followed the late Prof. T.B. Wood in directing the Institute of Animal Nutrition. He became in succession, Fellow, Tutor, Dean, and finally, in 1940, Vice-Master of Christ's College. He was a Proctor in 1911–12 and afterwards served on the Council of the Senate. Marshall was an original member of the Council of Management of the *Journal of Endocrinology* and was one of the four honorary members of the Society for Endocrinology. For many years he was an editor of the *Journal of Agricultural Science*, and finally became principal editor. Marshall's scientific distinctions included D.Sc. (Edin.), Sc.D. (Cantab.), D.Sc. (Hon. Manch.), LL.D. (Hon. Edin.), F.R.S. (1920), C.B.E. (1933), Council of the Royal Society (1933–5), Baly Medal of the Royal College of Physicians (1935), Croonian Lecturer (Royal Society) 1936, and Royal Medal (Royal Society) 1940.

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A BIOGRAPHICAL NOTE PREPARED BY *G. E. LAMMING*

(for the Fourth Edition)

PROFESSOR SIR ALAN PARKES
CBE, FRS.

Sir Alan Parkes' association with F.H.A. Marshall began when he enrolled as an undergraduate at Christ's College, Cambridge and Marshall became his tutor. Parkes describes this first association with Marshall as no more than formal since he preferred to avoid undue contact with those in authority, no doubt prompted by previous experience at school and in the army. Parkes read agriculture, obtaining a pass degree in 1921 and an MA in 1924. After graduating in 1921 he applied for a position at the Shirley Cotton Research Institute in Manchester which (fortunately for reproductive physiology) he failed to obtain. However, fortified by his father's encouragement and support he entered Manchester University as a PhD student in the Zoology Department to research on the mammalian sex ratio. During this period Parkes accepted a request from Marshall to help him with indexing the second edition of MPR which was published in 1922. No doubt Marshall had been impressed, not so much by Parkes' enthusiasm as an undergraduate, but by the initiative he had shown in carrying out a literature search for his first published paper on 'Sex heredity', which appeared in *Science Progress* in 1921.

At Manchester, Parkes completed his PhD in two years having A.V. Hill as an internal examiner and Marshall as the external. The inclusion of A.V. Hill was fortunate for it resulted in Parkes moving with Hill, now a Professor, to University College, London, where Parkes remained until 1931, holding a

series of three research fellowships. Here he co-operated in researches with a number of famous contemporaries including Jack (later Sir Jack) Drummond, Professors F.W.R. Brambell, Guy Marrion and Solly (later Lord) Zuckerman. From 1932 to 1961 Parkes was a member of the MRC's National Institute of Medical Research, first at Hampstead and later at Mill Hill. He was responsible, with others, for organizing a number of research programmes in reproductive endocrinology, many of which had major influences on developments in reproductive physiology as applied to agriculture. Detailed research on important topics included the induction and control of ovulation and lactation, and the cryo-preservation of animal tissues. In 1961 Parkes returned to Cambridge, fittingly as the first holder of the newly created Mary Marshall Chair in the Physiology of Reproduction, established under the terms of Marshall's will. Here the nature of his work changed, for the Marshall position was vastly different from his previous MRC post; it lacked the fabric for Parkes to continue his detailed researches. Nevertheless, the change produced benefits which history may well judge of equal merit, for Parkes continued to influence the course of reproductive physiology by virtue of major roles in organizing societies and scientific journals including the Society for the Study of Fertility, its associated *Journal of Reproduction and Fertility*, and also the *Journal of Biosocial Sciences*, acting for a number of years in addition as executive editor for both journals. The following is abstracted (by kind permission of the publishers, A & C Black (Publishers) Limited) from his entry in the 1990 edition of *Who's Who*.

PARKES, Sir Alan (Sterling), Kt 1968; CBE 1956; FRS 1933; MA, PhD, DSc, ScD; Fellow of Christ's College, Cambridge, 1961–69, Hon. Fellow 1970; Fellow of University College, London; Chairman, Galton Foundation, since 1969; *b* 1900; *y s* of E. T. Parkes, Purley; *m* 1933, Ruth *d* of Edward Deanesly, FRCS, Cheltenham; one *s* two *d*. *Educ*: Willaston School; Christ's College, Cambridge; BA Cantab, 1921, ScD 1931; PhD Manchester, 1923; Sharpey Scholar, University College, London, 1923–24; Beit Memorial Research Fellow, 1924–30; MA Cantab 1925; Schäfer Prize in Physiology, 1926; DSc London 1927; Julius Mickle Fellowship, University of London, 1929; Hon. Lecturer, University College, London, 1929–31; Member of the Staff of the National Institute for Medical Research, London, 1932–61; Mary Marshall Prof. of the Physiology of Reproduction, Univ. of Cambridge, 1961–67, Professor Emeritus 1968. Consultant, Cayman Turtle Farm Ltd, Grand Cayman, BW1, 1973–. Foulerton Student of the Royal Society, 1930–34. Mem., Biol. and Med. Cttee, Royal Commn on Population, 1944–46. President: Section of Endocrinology, Roy. Soc. Med., 1949–50, Section of Comparative Medicine, 1962–63; Section D Brit. Assoc. for the Advancement of Science, 1958; Eugenics Soc., 1968–70; Inst. of Biology, 1959–61; Assoc. of Scientific Workers, 1960–62. Chairman: Soc. for Endocrinology, 1944–51; Soc. for Study of Fertility, 1950–52, 1963–66; Nuffield Unit of Tropical Animal Ecology, 1966–69; Breeding Policy Cttee, Zool Soc. of London, 1960–67; Scientific Adv. Cttee, Brit. Egg Mkting Bd, 1961–70. Mem. Adv. Cttee on Med. Research of the WHO, 1968–71. Executive Editor, Jl Biosocial Science, 1969–; Sec., Jls of Reproduction & Fertility Ltd, 1970–76. Consultant, IPPF, 1969–79. Cameron Prize, 1962; Sir Henry Dale Medal, Soc. for Endocrinology, 1965; John Scott Award (jtly with Dr A. U. Smith and Dr C. Polge), City of Philadelphia, 1969; Marshall Medal, Soc. Stud. Fert., 1970; Oliver Bird medal, FPA, 1970. *Publications*: The Internal Secretions of the Ovary, 1929; Sex, Science and Society, 1966; Patterns of Sexuality and Reproduction, 1976; Off-beat Biologist, 1985; Biologist at Large, 1988; papers on the Physiology of Reproduction, on Endocrinology and on the behaviour of living cells at low temperatures in Jl of Physiology, Proc. Royal Society and other scientific jls. Ed. Marshall's Physiology of Reproduction, 3rd edn, 1952, consultant 4th edn, 1994. *Address*: 1 The Bramleys, Shepreth, Royston, Hertfordshire SG8 6PY.

Professor Parkes died on July 17th 1990.

ACKNOWLEDGEMENTS

Fortunately, few will have experienced the volume of work involved in commissioning, editing and preparing a text of this size. It can only be completed by competent teamwork. My main task has been to persuade the team members to participate and then organize their work.

My special thanks goes initially to the contributors but also to their secretaries. It is well known that the best authors are busy individuals heavily committed both to their research and to allied commitments, (including travel abroad). Thus, many of my queries concerning the texts are handled initially (and, in some cases, entirely) by secretaries. I applaud their tenacity and tact, and also their skill, especially in checking reference citations. I owe them a special vote of thanks.

Mrs Pamela Blythe completed the initial secretarial work prior to her move to a senior managerial post elsewhere. The main bulk of the editorial work was completed by Mrs Linda Faillace during her husband's (Dr L. Faillace) postdoctoral research work in this department. I acknowledge her skill in the preparation of texts for printing and thank her husband and family for their forebearance in tolerating the many hours of work she completed, both in the office and at home. I also thank Mrs Monica Bagshaw for providing additional secretarial help, and Dr Karen Shelton for her contribution to chapter 2.

I again thank collectively the advisers to the Fourth Edition. Professor Parkes assisted, prior to his death, in the initial plans. Several of the advisers were helpful not only in planning the volume, but also in the detailed discussions involving our move to publication by Chapman & Hall. I acknowledge the commitment, skill and speed of the Chapman & Hall staff (Miss Rachel Young, Mr Martin Tribe and Mr Jeremy Macdonald) in publishing this volume.

Finally, I reserve special praise for those who have completed the indexing of volume 3 with a total of about 10 000 reference citations. What a pleasure it is to have the continued help of Dr Barbara Weir as an adviser, and to take advantage of her skill in the preparation of the subject index. Her knowledge of world literature in reproductive physiology, gained from years of experience as Editor of the *Journal of Reproduction and Fertility*, is invaluable. I am sure many who use these texts, and who know Barbara personally, will support my expression of gratitude.

The author index was ably and generously completed by my young staff colleagues. (They may comment that they had little choice since I organize their work schedule). I am most fortunate to have young staff colleagues who cheerfully tackled these time-consuming chores, and I record my sincere appreciation to Misses Morag Batten, Sarah Machin, Jane Plowright and Alison Clarkson.

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THE OVARIAN EGG AND OVULATION 1

A. Tsafriri, R. Reich and A.O. Abisogun

Humbly dedicated to the memory of Professor Bernhard Zondek (1881–1966), one of the founders of reproductive endocrinology, and a pioneer of modern obstetrics and gynaecology, in Jerusalem, Israel.

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1.1 INTRODUCTION

The production of a fertilizable ovum is a protracted process spanning the embryonic, post-natal, prepubertal and sexually mature life of the mammalian female. In Figure 1.1 the life history of the rat ovum is depicted schematically on the background of ontogenetic development.

In brief, a short period of mitotic activity of oogonia is followed by their transformation into primary oocytes which embark on meiosis. The meiotic process is subject to multiple stop–go controls. The term 'oogenesis' has been used in the literature to describe (i) the transformation of oogonia into oocytes (Franchi *et al.*, 1962; Zuckerman and Baker, 1977; Peters and McNatty, 1980) and (ii) the entire process of the formation and maturation of the female germ cell (Baker, 1982). This wider definition is similar to the term spermatogenesis and is presently used more frequently and will be adopted here. We shall use the term 'oocytogenesis' (Kennelly and Foote, 1966; Siracusa *et al.*, 1985) to indicate the transformation of oogonia into oocytes and initiation of meiosis.

From early stages of germ cell life a close association with somatic cells of the ovary is established (Mitchell and Burghardt, 1986). Oocytes which do not become enclosed within granulosa cells, and thus incorporated into primordial follicles, usually degenerate (Ohno and Smith, 1964). Furthermore, the growth of the oocyte and of the follicle are closely coordinated and the ovulatory stimulus induces changes both in the oocyte and in the somatic compartment of the follicle. Therefore, normal development of the ovum, especially the later stages of its growth and maturation, can be properly discussed only within the framework of follicular physiology.

In most mammalian species meiotic maturation is initiated during prenatal life or shortly after birth, but the process is arrested (**first meiotic arrest**) at the diplotene stage. In rodents, the chromosomes become diffuse and the stage is referred to as the dictyate stage of the prophase of first meiotic division (Figure 1.2).

The meiotic arrest is maintained throughout oocyte and follicular development to the Graafian follicle stage. *In vivo* the meiotic process is resumed only in fully grown oocytes in follicles responding to the preovulatory surge of gonadotrophins (Ayalon *et al.*, 1972) or in follicles undergoing atresia (reviewed by Tsafriri and Braw, 1984). At ovulation, in most mammalian species, a secondary oocyte arrested at the metaphase of the second meiotic division (**second meiotic arrest**) is released. The second meiotic division is completed only after fertilization or a parthenogenetic stimulus and evidenced by abstriction of the second polar body.

The maintenance of first meiotic arrest throughout oocyte and follicular growth and its termination following the preovulatory surge of gonadotrophins has been the subject of many recent studies. While, within the follicle, resumption of meiosis is strictly dependent upon hormonal stimulation, explantation of ova from Graafian follicles results in their spontaneous maturation *in vitro* (Pincus and Enzmann, 1935). Subsequent studies demonstrated that the ability of oocytes to resume meiosis spontaneously, usually referred to as 'meiotic competence', is acquired only during later stages of oocyte growth and is related to follicular development (Szybek, 1972; Erickson and Sorensen, 1974; Bar-Ami and Tsafriri, 1981).

In this chapter we shall review the life history of the mammalian ovum throughout embryonic development to ovulation and relate it to follicular development. Attention will be given to the following subjects: (i) the development of meiotic competence; (ii) mechanisms involved in the maintenance of meiotic arrest; (iii) the ovulatory changes induced by the surge of gonadotrophins, in-

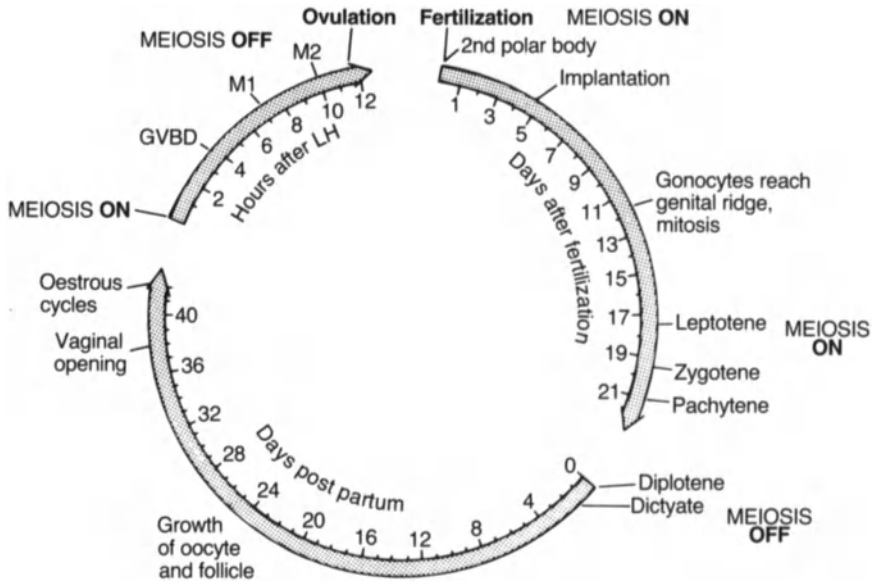

Figure 1.1 Schematic presentation of oogenesis and its multiple stop–go controls in the rat. (From Lindner *et al.*, 1980.)

cluding resumption of meiotic maturation and follicular rupture.

Most of the investigations to be reviewed have been performed using laboratory animals, such as mice, rats and rabbits. Studies carried out on other species will be referred to when pertinent.

1.2 THE PRIMORDIAL GERM CELLS

The origin and biology of mammalian primordial germ cells (PGCs) have been reviewed recently (Gondos, 1978; Hardisty, 1978; Heath 1978a; Eddy *et al.*, 1981; McLaren, 1981, 1983a; Siracusa *et al.*, 1985; Byskov, 1986).

Experimental evidence for the extragonadal origin of germ cells in mammals was obtained by Everett (1943). He demonstrated that transplantation of genital ridges from 10.5- to 14-day-old mouse embryos beneath the kidney capsule of adult mice resulted in normal differentiation of the transplanted gonads. By contrast, genital ridges explanted from younger mouse embryos, between 9.5 and 10 days, before germ cells could be discerned, did not develop germ cells.

The PGCs were first recognized at the base of the allantois of human embryos (Witschi, 1948). This early observation was later extended using PGCs' high alkaline phosphatase activity as a marker (McKay *et al.*, 1953). In the mouse PGCs were identified in the yolk sac endoderm and at the root of the allantois as early as the eighth day of gestation, around the time of the appearance of the first somite (Chiquoine, 1954; Mintz and Russell, 1957; Jost and Prepin, 1966; J.M. Clark and Eddy, 1975). The progenitors of PGCs in the mouse were traced 24 h earlier to a small cluster of alkaline phosphatase-positive cells just posterior to the primitive streak in the extraembryonic mesoderm (Ginsburg *et al.*, 1990). The germ cell lineage derives from the epiblast (embryonic ectoderm; McLaren, 1983a; Gardner *et al.*, 1985) and not from the yolk sac endoderm as previously believed. Blastomeres of a two-cell embryo (Tarkowski, 1959), or at least three blastomeres of a four-cell embryo (Kelly,

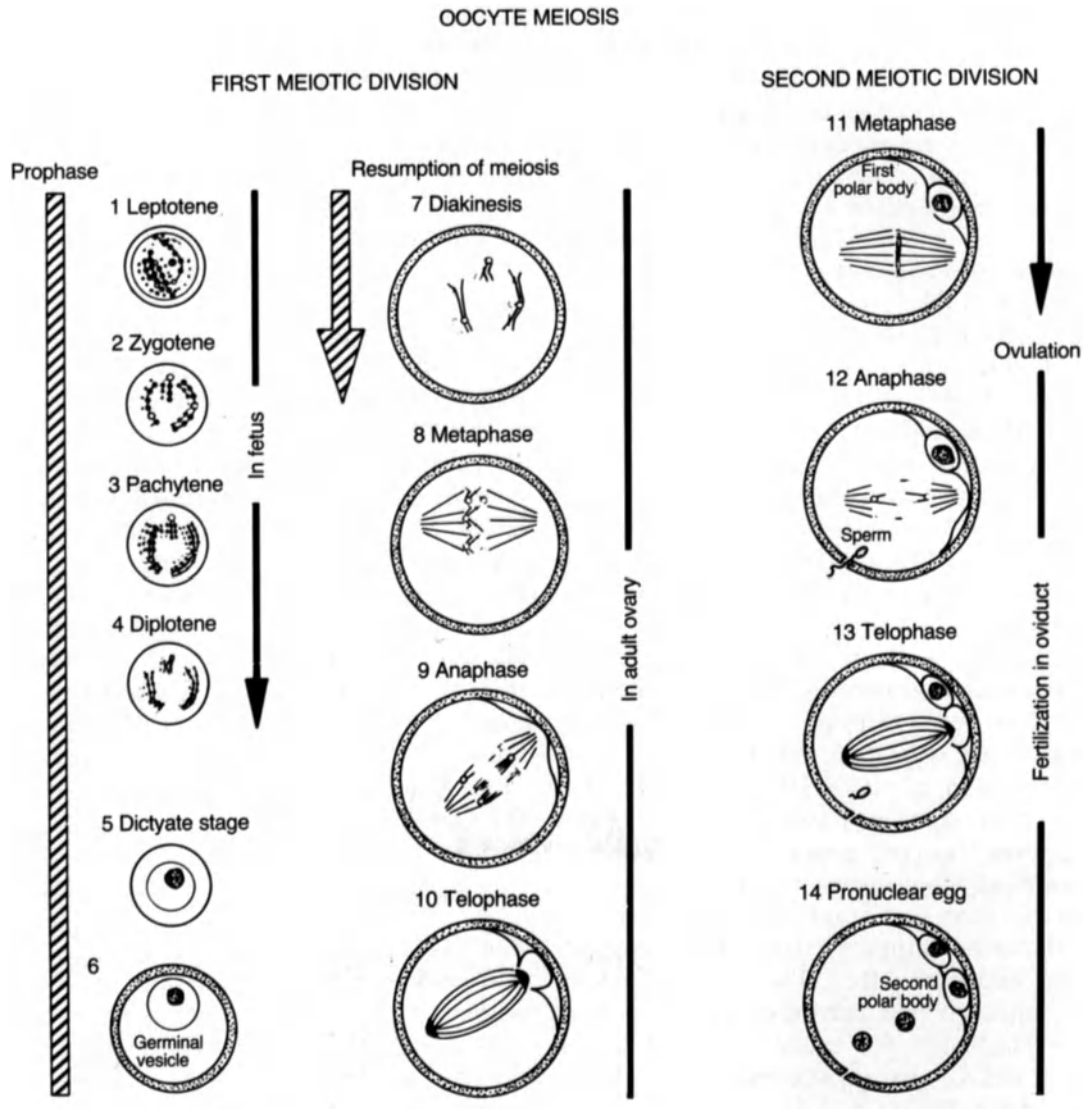

Figure 1.2 Oocyte meiosis. For simplicity, only three pairs of chromosomes are depicted. (1–4) Prophase stages of the first meiotic division that occur in most mammals during fetal life. At zygotene (2) the homologous maternal and paternal chromosomes begin to pair. At pachytene (3) they are paired along their entire length, thus forming bivalents. During pachytene, each homologue cleaves longitudinally to form two sister chromatids, so that each bivalent forms a tetrad. During this stage, interchange of genetic material between maternal and paternal chromatids may occur by crossing over. At diplotene (4), the chromosomes begin to separate, remaining united at the chiasmata. The meiotic process is arrested at this stage (first meiotic arrest), and the oocyte enters the dictyate stage. When meiosis is resumed, the first maturation division is completed (7–11). Ovulation usually occurs at the metaphase II stage (11) (second meiotic arrest), and the second meiotic division (12–14 is completed in the oviduct following sperm penetration. (From Tsafriri (1978) with kind permission of Plenum Publishing, New York.)

1977) or at least two cells in fifth day primitive ectoderm (Gardner *et al.*, 1985), can give rise to both somatic cells and functional germ cells when transplanted into host blastocysts. Thus, the segregation of the germ line occurs after day 5 of mouse embryonic development (Gardner *et al.*, 1985) and before day 7 (see discussion in Eddy *et al.*, 1981).

In the mouse the PGCs migrate from the yolk sac and allantois (8- to 9-day embryos), throughout the hindgut epithelium (9- to 10-day embryos) and dorsal gut mesentery (10- to 12-day embryos), into the developing gonads (beginning on day 10.5) (Chiquoine, 1954; Mintz, 1959; Ozdzenski, 1967). The migration route of PGCs is similar in other mammalian species examined.

The precise mechanism(s) involved in the migration of PGCs to the gonad are poorly understood. The identification of pseudopodia-like projections in mouse, rat and human PGCs (Jeon and Kennedy, 1973; Spiegelman and Bennet, 1973; Zamboni and Merchant, 1973; Chung, 1974; Eddy, 1974; Eddy and Clark, 1975; Fujimoto *et al.*, 1977; Miyayama *et al.*, 1977; Heath, 1978b; Lin *et al.*, 1982; Kuwana and Fujimoto, 1983) supports active amoeboid movement. Identification of microfilaments in the cytoplasmic processes of migrating mouse PGCs lends further support to this notion (Spiegelman and Bennet, 1973). Morphogenetic movement of the surrounding tissues was also suggested for translocation of PGCs (Jeon and Kennedy, 1973; Snow, 1981; Snow and Monk, 1983).

The direction of PGCs' movement is attributed to contact guidance or differential adhesion of the surrounding tissue. Chemotaxis, i.e. movement directed towards the developing gonads, which secrete a chemical attractant, was suggested by Witschi (1948). Transplantation of mouse hindguts into developing chick embryos resulted in invasion of mouse PGCs into the host gonad (Rogulska *et al.*, 1971), thus supporting the suggested chemotactic action of the gonad. PGCs from 12.5- to 15.5-day-old mouse embryos specifically adhered to somatic cells of mature gonads, but not other embryonal and adult cell types (De Felici and Siracusa, 1985).

During their migration and settling in the gonad the PGCs divide mitotically. When first recognized, the number of PGCs in mouse embryos is less than 100, it reaches 4000 on day 12 (Mintz and Russell, 1957), and 25 000 by day 13.5 when colonization of gonads is completed (Tam and Snow, 1981). A doubling time of 16 h was estimated for mouse PGCs during migration (Tam and Snow, 1981).

The number of PGCs may be influenced by genetic defects and experimental manipulation. Thus, in homozygous embryos for the mutant genes *Steel* (S1) or *White-spotting* (W), the number of PGCs reaching the gonad is markedly reduced (Bennet, 1956; Mintz and Russell, 1957). Administration of busulphan (Merchant-Larios, 1975; Merchant-Larios and Coello, 1979) or mitomycin C (Tam and Snow, 1981) to pregnant mice or α-irradiation of pregnant rats (Beaumont, 1966) decreased the number of PGCs.

1.3 OOGONIA

The PGCs colonizing the developing ovary and undergoing mitotic divisions are termed oogonia. When mitotic divisions cease and the cells enter meiosis they are termed oocytes. As already indicated, very often the transformation of oogonia into oocytes was referred to as 'oogenesis'. Here, we shall use the term 'oocytogenesis' instead.

Morphologically oogonia are similar to PGCs. They are somewhat more rounded when compared with PGCs, which are amoeboid in appearance (Gondos, 1978). In measurements using histological sections (probably underestimates due to dehydration), oogonia in dog and monkey ovaries had a diameter of 13 μm (Andersen and Simpson, 1973; Gulyas *et al.*, 1977). In ovaries

embedded for electronic microscopy in plastic, oogonial diameter of 22 μm was reported in cat ovaries (Amselgrüber, 1982) and 19 μm in the human ovary (Dvoràk and Tesàrik, 1980). The major distinction between PGCs and oogonia is in their location. Oogonia are located within more or less distinct germ cells cords or in clusters (Gondos, 1978; Byskov, 1986). In many species examined oogonia are connected by intercellular bridges (see Gondos, 1978, and Byskov, 1986, for references). They are formed by incomplete cytokinesis at telophase. The intercellular bridges are thought to serve for intercellular communication and transfer of metabolites and regulatory substances. Synchronization of mitoses within conjoined cells has been demonstrated in oogonia in the mouse (Ruby *et al.*, 1969), rabbit (Peters *et al.*, 1965), cow and sheep (Rüsse, 1982, 1983).

1.3.1 OOGONIAL PROLIFERATION AND DEGENERATION

The oogonial stage of development is characterized by mitotic activity. In some species the oogonial stage occurs entirely during the prenatal life (rat, mouse, guinea pig, cow, sheep, pig, rhesus monkey and human), while in others it extends into the neonatal life (cat, rabbit, ferret, mink, vole and hamster, see Table 1.1).

Analysis of oogonial divisions revealed resemblance with spermatogonial divisions in the sheep (Mauléon, 1967) and prespermatogonia in the rat (Hilscher and Hilscher, 1978). The relatively high mitotic index of oogonia results in marked increase in their number (rat, Beaumont and Mandl, 1962; mouse, Tam and Snow, 1981; guinea pig, Ioannou, 1964; rabbit, Chretien, 1966; pig, Black and Erickson, 1968; human, Baker and O, 1976).

The proliferation of oogonia is accompanied by degeneration of large numbers of oogonia. Oogonia degenerate at interphase or during mitosis. Some authors (Beaumont and Mandl, 1962; Ioannou, 1964), used the term 'atretic divisions' for the latter. Degeneration of oogonia does not include conspicuous changes in somatic cells of the ovary. We prefer to use the term 'atresia' only

Table 1.1 Time and duration of ooctytogenesis in different mannals. (From Peters and McNatty, 1980, by permission of Granada Publishing, London).

	Age of post-partum fetus (days)		
Species (ref [a])	*Onset of meiosis*	*Arrest of meiosis*	*Duration*
Rat[1]	17	18	2 days
Mouse[2]	13	16	4 days
Guinea pig[3]	30	50	3 weeks
Pig[4]	30	100	10 weeks
Ewe[5]	52	82	4 weeks
Cow[6]	75	160	12 weeks
Monkey[7]	2 months	At birth	4 months
Human[8]	2 months	7 months	5 months
Golden hamster[9]	Day of birth	6	7 days
Rabbit[10]	1	10	10 days
Ferret[11]	6	14–20	8–14 days
Cat[12]	40–50	8	18 days

[a] References: [1]Beaumont and Mandl (1952); [2]Borum (1961); [3]Ioannou (1964); [4]Black and Erickson (1968); [5]Mauléon and Mariana (1976); [6]Erickson (1966); [7]Baker (1966); [8]Baker (1963); [9]Weakley (1967); [10]Peters *et al.* (1965); [11]Deanesly (1970); [12]von Winiwarter and Sainmont (1909).

for elimination of antral follicles by other means rather than ovulation (Tsafriri and Braw, 1984). Oogonial degeneration, too, seems to appear in clusters of oogonia (Gondos and Zamboni, 1969; Gondos, 1973).

1.3.2 PREMEIOTIC DNA SYNTHESIS

In several mammalian species examined, the mitotic activity reaches its peak just before the start of meiosis (mouse, Hilscher and Hilscher, 1981; Peters and Crone, 1967; rat, Hilscher *et al.*, 1974; guinea pig, Ioannou, 1964; sheep and cattle, Rüsse, 1982; pig, Black and Erickson, 1968; monkey, Baker, 1966; human, Baker, 1963).

In all mitotically proliferating cells, DNA is synthesized during the S phase of the interphase of the cell cycle. Likewise, in mitotically dividing germ cells, PGCs or oogonia, DNA is synthesized during interphase preceding division. In addition, there is a phase of DNA synthesis during the interphase preceding the initiation of meiosis and transformation of oogonia to oocytes. This transition is probably initiated during the G_1 phase of the cell cycle, and the subsequent S phase is referred to as 'premeiotic S phase' or 'premeiotic DNA synthesis'. This premeiotic DNA synthesis may extend from interphase to leptotene stage of the prophase of the meiotic division as observed in rabbit oocytes by autoradiography (Peters *et al.*, 1965). The duration of this phase is similar (9–12 h) in mouse and rabbit oocytes (Lima-de-Faria and Borum, 1962; Crone *et al.*, 1965; Kennelly *et al.*, 1970) and it coincides with the transformation of oogonia to oocytes, or oocytogenesis. Thus, multiple injections of tritiated thymidine between days 13 and 15 in mouse embryos and during the neonatal period in rabbits result in labelling of almost all oocytes in the adult animal (Borum, 1966; Kennelly and Foote, 1966). These experiments provided the conclusive evidence for the continuity of the germ cell line from oogonium to the ovum and refuted the suggestion of neoformation of germ cells after this period of DNA synthesis. For further discussion of these two alternatives see the reviews by Franchi *et al.* (1962) and Zuckerman and Baker (1977).

Nevertheless, in some species of the Lemuroid family, groups of oogonia persist in the adult ovary. These oogonia can be labelled by tritiated thymidine and may transform into oocytes, suggesting oocytogenesis in the adult life (Herlant, 1961; Ioannou, 1967; Anand Kumar, 1968). It is doubted whether these oocytes enter the phase of follicular growth (David *et al.*, 1974).

1.4 THE OOCYTE

The first meiotic maturation is characterized by essentially universal and orderly sequenced changes in chromosome structure during the protracted prophase. These were described at the turn of the century in histological preparation of rabbit, human and cat ovaries (von Winiwarter, 1901; von Winiwarter and Sainmont, 1909). In later studies squash preparations or spreads (Ohno *et al.*, 1961, 1962; Luciani *et al.*, 1974; Bakken and McClanahan, 1978; Speed, 1982) and electron microscopy were employed (Franchi and Mandl, 1962; Tsuda, 1965; Baker and Franchi, 1967a, 1972; Comings and Okada, 1972; Holm and Rasmussen, 1977; Speed, 1982).

During leptotene, dispersed chromatin condenses progressively into regular threads containing a proteinaceous axial core. In zygotene, the pairing of homologous chromosomes starts. The synaptonemal complex forms with the appearance of its central element between closely opposed chromosomal axial cores, in effect zipping them up. Pairing is completed during early pachytene, when the chromosomes reach their most compact state and are short and thick. Electron microscopic studies revealed lateral projections of the chromatin fibres (Baker and Franchi, 1967a), and these become more pro-

nounced in early diplotene chromosomes. During diplotene the homologous chromosomes separate, their axes elongate and the chiasmata can be recognized. Thus, during the meiotic prophase exchange of paternal and maternal genes is made possible by crossing over.

In growing oocytes of many animal species producing large eggs, with notable examples in anuran and urodele amphibia, the pachytene–early diplotene chromosomes have large lateral loops with appearance of a test-tube brush or lampbrush and hence are referred to as 'lampbrush chromosomes'. These loops may reach several micrometres in length (Müller, 1974). These loops represent very active transcription units. The chromosomes of mammalian prophase oocytes cannot be considered as lampbrush chromosomes on the basis of their morphological and functional properties (see Bachvarova, 1985).

The duration of leptotene stage is between 3 and 6 h in the mouse and between 3 and 8 h in the rabbit. Zygotene is completed in the mouse within 12–14 h, and within 16–44 h in the rabbit. Pachytene takes more than 60 h in the mouse and lasts up to 10 days in the rabbit (mouse, Lima-de-Faria and Borum, 1962; Crone *et al.*, 1965; rabbit, Peters *et al.*, 1965; Kennelly *et al.*, 1970). At the diplotene stage the meiotic process is arrested, to be resumed only upon atresia or in preovulatory follicles after the surge of gonadotrophins. Hence, diplotene is by far the longest stage of meiotic prophase. In the human it may last about 50 years, i.e. from the initiation of diplotene in the fetus to the last ovulation in adult life. In many mammalian species, including the cat, cow, rabbit and human, the chromosomes retain their diplotene configuration throughout meiotic arrest (Ohno *et al.*, 1962; Baker and Franchi, 1967b; Zybina, 1975). Conversely, the diplotene chromosomes of rodents, such as the rat, mouse and hamster, become diffuse and assume the appearance of elongated fuzzy threads occupying most of the nuclear volume and are currently referred to as dictyate. This term is, therefore, equivalent to diffuse diplotene.

During the meiotic prophase the female germ cell undergoes morphological changes though maintaining spherical shape of the cell and the nucleus (see Gondos, 1978). The nuclear membrane is sustained throughout meiotic prophase. At the leptotene, the chromosomes attach to the inner nuclear membrane. These attachment sites seem to be crucial for the formation of synaptonemal complexes (Baker and Franchi, 1972; Rasmussen and Holm, 1980). The nucleolus remains compacted and relatively small until diplotene, when it enlarges and occasionally assumes complex reticular structure (Gondos *et al.*, 1971). In the human oocyte, multiple micronuclei associated with the heterochromatin appear during this period (Stahl *et al.*, 1973, 1975). The number and size of cytoplasmic organelles increases during meiotic prophase, but these changes differ among species (Gondos, 1978).

1.4.1 REGULATION OF MEIOSIS INITIATION

Meiosis is initiated much earlier in female germ cells than in male ones. Thus, in the female mouse, all germ cells enter the prophase of meiosis nearly a week before birth, while in the male the first meiotic germ cells do not appear in the testis until a week after birth. Two hypotheses were raised to account for the early onset of meiosis in female germ cells: local influence of the environment or a preprogrammed event. At the present it is impossible to decide between these two alternative hypotheses because of conflicting experimental observations and inadequacies of the available *in vitro* systems (see McLaren, 1984; Byskov, 1986).

The observation that meiosis starts in the central parts of the ovary, close to the mesonephric tissue (see Gondos, 1978; Byskov, 1986), gave rise to the hypothesis that cells of

mesonephric origin trigger the initiation of meiosis (Byskov, 1975). Transplantation experiments involving fetal mouse ovaries resulted in initiation of meiosis only when they were explanted with their attached mesonephric tissue (Byskov, 1975). Furthermore, meiosis could be induced in fetal undifferentiated mouse testes cultured in close proximity with ovaries containing the attached mesonephros (Byskov and Saxén, 1976). It was suggested that the mesonephric–ovarian complex, rete ovarii, secretes a meiosis-inducing substance (MIS) which is responsible for the initiation of meiosis (Byskov, 1978a). Similar results were obtained in fetal hamster ovaries transplanted *in vitro* (O and Baker, 1976; Fajer *et al.*, 1979).

The delay in the initiation of meiosis by male germ cells in the testis was ascribed to the action of a meiosis-preventing substance (MPS) and, conversely, the initiation of meiosis at puberty to either cessation of MPS secretion (Byskov and Saxén, 1976; Grinsted *et al.*, 1979) or to an increase in MIS secretion (Parvinen *et al.*, 1982).

In cultures of fetal rat ovaries initiation of meiosis was not dependent on the presence of mesonephric tissue (Stein and Anderson, 1979; Byskov, 1986) and no evidence for MIS activity was obtained in co-cultures of fetal mouse gonads (Evans *et al.*, 1982). Finally, in sheep and in the mouse, germ cells located in the adrenals were in meiotic prophase in both female and male fetuses (Upadhyay and Zamboni, 1982; Zamboni and Upadhyay, 1983). These findings were confirmed and extended in mouse ectopic germ cells. In female fetuses both ovarian and ectopic germ cells entered meiosis. Conversely, in male fetuses only testicular germ cells did not enter meiosis, but many germ cells in extratesticular stroma entered meiosis at days 18 and 19 of development (McLaren and Hogg, cited in McLaren, 1984; Francavilla and Zamboni, 1985). These latter findings seem to suggest that the autonomous differentiation potential of the mammalian germ cells is predominantly female and this is superseded by testicular influence.

The reactivation of the second X chromosome in female germ cells occurs prior to initiation of meiosis (Monk and McLaren, 1981). Nevertheless, reactivation of X chromosome occurs also in sex-reversed (Sxr) XX testes and the germ cells enter meiosis well after birth (McLaren and Monk, 1981). Reactivation of X chromosome, or chromosome constitution, therefore, does not seem to play a decisive role in the initiation of meiosis (see McLaren, 1983b).

In conclusion, experimental evidence for predetermined female-type early initiation of meiosis and for local MIS and MPS activity has been forwarded. Nevertheless, the precise interaction of any or all of these factors during ovarian development of various mammalian species remains obscure. Initial data seem to suggest that MIS is of steroid-like nature (Yding Andersen *et al.*, 1981). Work aimed at the chemical characterization and purification of MIS and MPS is especially needed. This would allow direct experimentation and determination of the mechanism(s) involved in regulation of initiation of meiosis.

1.4.2 DEGENERATION OF OOCYTES

Onset of meiosis is accompanied by further increase in germ cell degeneration. The proportion of germ cells surviving degeneration ranges from only 5% in the cow (Erickson, 1966) and human (Baker, 1963) to 50% in the pig (Black and Erickson, 1968). Thus, the maximal number of germ cells is reached upon oogonial mitoses, and from this fetal peak there is continuous depletion in their number throughout embryonic development, infancy and sexually mature life (Figure 1.3).

Germ cells degenerate at all stages of development, but quantitatively three major 'waves' of degeneration have been reported in the human fetal ovary. These 'waves'

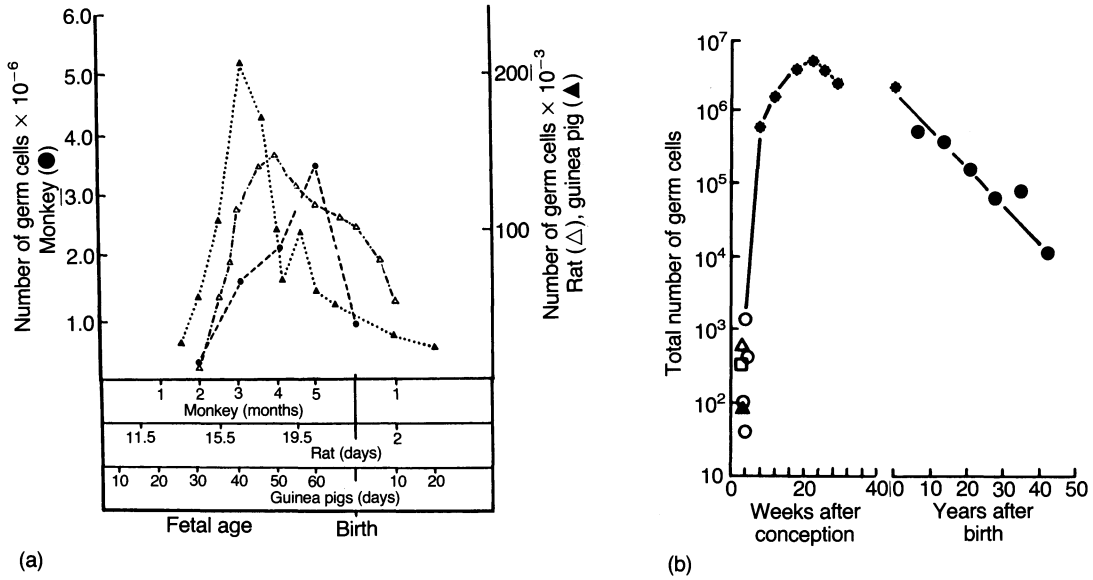

Figure 1.3 The number of germ cells in (a) the ovaries of fetuses and neonates in monkey, rat and guinea pig (from Baker, 1972) and (b) in the human female. (From Siracusa *et al.*, 1985.)

affect mitotically active oogonia, as well as oocytes in pachytene and diplotene stages of meiotic prophase (Baker, 1963). Degenerating zygotene and pachytene oocytes have been referred to as 'Z' cells (Beaumont and Mandl, 1962). Degenerating cells are characterized by conspicuous condensation of chromosomes and are phagocytosed by somatic ovarian cells (see details and references, Gondos, 1978).

Genetic factors play an important role in oocyte degeneration. Thus, X chromosome monosomy results in excessive degeneration of oocytes in early meiotic prophase in the human (Singh and Carr, 1966) and during fetal and neonatal development in the mouse (Burgoyne and Baker, 1981).

The role of extragonadal hormones in progression of meiosis and maintenance of primary oocytes is obscure. In fetal hypophysectomized rhesus monkeys germ cell degeneration was increased greatly. The poor development of the rete ovarii or their absence was suggested as a possible reason for germ cell degeneration, rather than direct dependence on fetal pituitary hormones (Gulyas *et al.*, 1977). Likewise, thymectomy of rhesus monkey fetuses resulted in reduction in the number of oogonia and meiotic oocytes in ovaries observed at term, in addition to increased degeneration of preantral and antral follicles (Healy *et al.*, 1985). The mechanisms involved remain obscure. As thymectomy raised fetal plasma gonadotrophin levels, it is possible that endocrine as well as immune mechanisms are involved in germ cell differentiation and maintenance. Recently, a marked increase in the progression of meiosis in rabbit fetal ovaries cultured *in vitro* with pituitaries was observed (Mazur and YoungLai, 1986). The higher FSH levels in female fetuses at the time of initiation of meiosis, as compared with male fetuses of the same age, in sheep (Mauléon and Reviers, 1969) and rabbits (YoungLai *et al.*, 1981) support the notion that hypophyseal secretions may be involved in the progression of meiosis and maintenance of meiotic germ cells. Nevertheless, it should be emphasized that meiosis proceeds, albeit in a smaller number of germ cells, even in the absence of pituitary. It is possible, therefore,

that pituitary secretions affect the number of germ cells indirectly through trophic action on somatic cells of the ovary.

1.5 OVARIAN DIFFERENTIATION AND FOLLICULOGENESIS

In the previous sections our discussion was focused on the ovarian germ cell compartment. Now the changes in the germ cells will be related with those occurring in the somatic compartment(s) of the ovary. Ovarian differentiation has been reviewed recently (Merchant-Larios, 1978; Peters, 1978; Pelliniemi and Dym, 1980; Peters and McNatty, 1980; Byskov, 1986; Byskov and Hoyer, 1987; Hirshfield, 1991), therefore, only a brief overview will be presented.

1.5.1 OVARIAN DIFFERENTIATION

In most mammalian species the most conspicuous early change associated with ovarian differentiation is the initiation of meiotic prophase (Peters, 1970). Nevertheless, in some species, such as the sheep, pig, cow and rabbit, initiation of meiosis is delayed (Mauléon, 1967, 1969) and, hence, a relatively long delay period separates the first manifestation of gonadal sex differentiation, i.e. recognizable testes in the male and the onset of meiosis in the female fetuses.

In species with delayed onset of meiosis, ovarian differentiation includes formation of well-defined sex cords containing germ cells (horse and cow, Y. Hashimoto and Eguchi, 1955a,b; Henricson and Rajakoski, 1959; cat, mink and ferret, Byskov, 1975; sheep, Zamboni *et al.*, 1979; pig, Pelliniemi and Lauteala, 1981) or formation of distinctive cortex and medulla (human and rabbit, Wartenberg, 1983; YoungLai and Byskov, 1983). By contrast, in species with early meiosis, like the mouse and the rat, there is no formation of distinct germ cell cords or organization of cortex–medulla (Byskov, 1978a). Further development of the ovary includes formation of follicles, folliculogenesis and the differentiation of steroid-producing cells.

1.5.2 FOLLICULOGENESIS

In the early stage of ovarian development, the nuclei of the oocytes lie closely together in nests with only partial separation by a plasma membrane. The nests of oocytes are usually surrounded by thin stromal cells (see Peters, 1978). As the oocytes reach diplotene stage, the plasma membrane is completed and the bridges between adjacent oocytes disappear. Thus, during ovarian differentiation the nests of oocytes are transformed from a cell syncytium into clusters of naked oocytes surrounded by somatic cells. The enclosure of diplotene oocytes with somatic cells is called folliculogenesis. The resulting small follicles consist of an oocyte, granulosa cells and a basement membrane. The time of follicle formation varies in different mammalian species (Table 1.2).

In most mammals folliculogenesis is limited to a well-defined period. Oocytes which do not become incorporated into follicles usually degenerate (Ohno and Smith, 1964).

Three tissues were suggested as the source of somatic cells of the follicle: (i) the coelomic or surface epithelium; (ii) the central blastema (or central stromal cells); (iii) the cells of the rete ovarii. The process of folliculogenesis starts at the innermost parts of the ovarian cortex. The granulosa cells of these follicles are initially continuous with rete ovarii, and only the formation of the basement membrane severs this continuity (mouse, Byskov and Lintern-Moore, 1973; Byskov and Rasmussen, 1973; rat, Stein and Anderson, 1979; dog, von Kölliker, 1898; rat, mink and ferret, Byskov, 1975). Furthermore, removal of the rete ovarii prior to the initiation of folliculogenesis prevents formation of follicles, while the removal of the surface epithelium has no effect on follicle formation in the mouse (Byskov, 1974; Byskov *et al.*, 1977).

Table 1.2 Ovarian differentiation and folliculogenesis in mammals

Species	*Gestation (days)*[a]	*Initiation of gonadal sex differentiation (days)*[a]	*Delay period (days)*[b]	*Follicular formation begins*	
				Post coitum (days)[c]	*Post partum (days)*[c]
Hamster	16	11–12	3		7
Mouse	19	12	0.5		2
Rat	21	13–14	3		1
Rabbit	31	15–16	15		14
Cat	65	30	12		11
Guinea pig	68	22–23	7	48	
Pig	114	26	18	64	
Sheep	150	35	20	65	
Cow	280	39	45	95	
Rhesus monkey	165	38	10	50	
Human	270	40–42	10[d]	56	

[a]From Gondos (1978).
[b]The period separating the time of gonadal sex differentiation, i.e. testicular differentiation which is observed earlier, and the beginning of meiosis in the ovary. From Byskov (1986).
[c]From Peters (1978). See the original publications for references.
[c]According to Gondos *et al.* (1986), oocytogenesis begins between 11 and 12 weeks of age, hence the delay period is > 35 days in the human.

Thus, a contribution of rete ovarii to follicle cells seems well established. The contribution of surface epithelium varies between species. In the human (van Wagenen and Simpson, 1965; Gondos, 1975), monkey (van Wagenen and Simpson, 1965), dog (Andersen and Simpson, 1973), cat (von Winiwarter and Sainmont, 1909), rabbit (Gondos, 1969) and guinea pig (Jeppesen, 1975) marked proliferation of the surface epithelium is observed at the time of follicle formation. Cords of epithelial cells extend from the surface and penetrate into the ovarian stroma. Some of these cords lose their connection to the surface, associate with oocytes and form small follicles. The ontogeny of thecal cells has been discussed recently by Hirshfield (1988).

Germ cells are required for folliculogenesis. Follicle formation is not observed in the absence of oocytes. The Turner's syndrome (XO constitution) in the human entails loss of germ cells and thus ovarian follicles are not formed. The resulting ovary, referred to as a 'streak' ovary, is devoid of follicles and normal endocrine activity (Singh and Carr, 1966; Morishima and Grumbach, 1968; Weiss, 1971; Reddoch *et al.*, 1986). Likewise, experimental destruction of germ cells during fetal development in the rat by busulphan abolishes folliculogenesis. The ovary is made up of a network of rete-like cords and tubules. Only when occasional oocytes survive do follicles form (Merchant-Larios, 1976). The small follicles form the pool from which follicles begin to grow throughout fetal, prepubertal and pubertal life.

1.6 FOLLICULAR GROWTH

1.6.1 CLASSIFICATION OF FOLLICLES

A classification of follicles first proposed for the mouse (T. Pedersen and Peters, 1968) and later adapted to the human (Peters *et al.*, 1978) has proved most useful for analysing follicular development (Figure 1.4).

Thus, four groups of follicles can be distinguished:

1. Small, non-growing follicles (also called **primordial** or **primary follicles**) consist of a small oocyte, a few flattened granulosa cells and a basement membrane. They lie in the outer cortex of the ovary and represent the pool of resting follicles.
2. The **preantral follicle** (also called **secondary**, **medium** or **growing** follicle) is the one that enters the growing phase. Its oocyte begins to grow and granulosa cells enlarge and multiple to form two or three layers around the oocyte. The zona pellucida forms and cells of the ovarian stroma organize into the theca layers.
3. The **antral follicle** contains a fully grown oocyte, several layers of granulosa cells, an antrum filled with fluid and, outside the basement membrane, well-differentiated theca layers.
4. The **large antral follicle** (also referred to as **Graafian follicle**), which reaches its maximum diameter and is fully responsible to the preovulatory surge of gonadotrophins.

1.6.2 FOLLICULAR GROWTH BEFORE PUBERTY

(a) Fetal life

In man and monkeys follicular growth starts during fetal life (van Wagenen and Simpson, 1965). The first small follicles appear during the fourth month of gestation and around the sixth month many preantral follicles can be located in the innermost part of ovarian cortex. Antral follicles often develop during the last 2 months of fetal life and the ovary of the newborn is often crowded with large antral follicles. However, these follicles show signs of degeneration or atresia and of irregular growth (Peters *et al.*, 1978).

Anencephalic human fetuses have a greatly reduced pituitary gland which contains only about 2% of the normal gonadotrophin content (Grumbach and Kaplan, 1973). Ovaries of such fetuses at term are

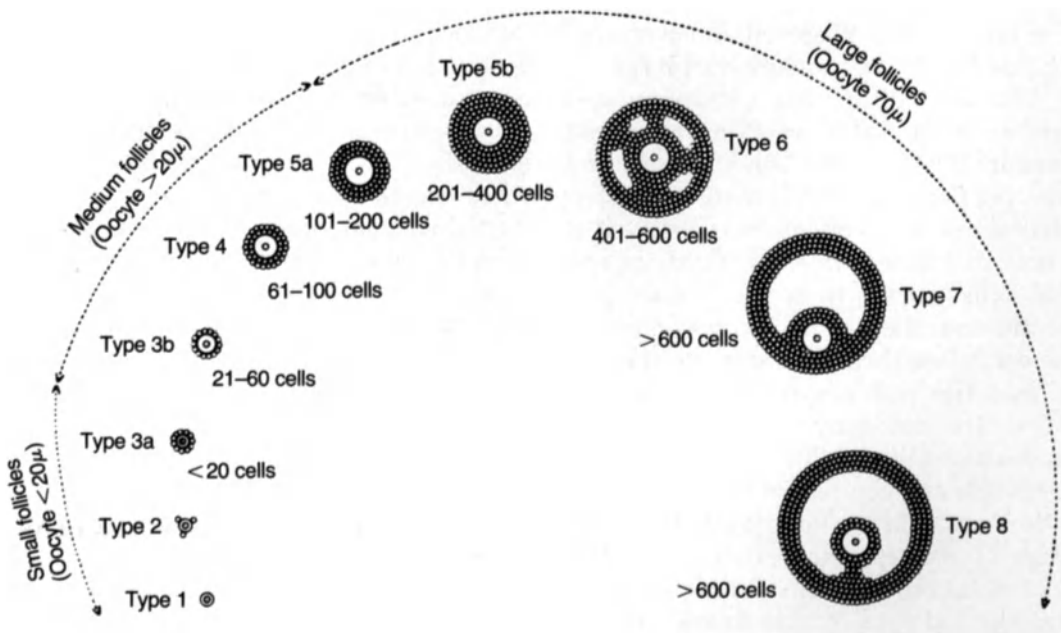

Figure 1.4 Classification of mouse follicles in the mouse ovary. (From Pedersen and Peters, 1968.)

small and contain very few preantral and no antral follicles (Baker and Scrimgeour, 1980). Also, hypophysectomy *in utero* of rhesus monkeys resulted in impaired growth of follicles (Gulyas *et al.*, 1977). These studies suggest that gonadotrophins of the fetal pituitary are involved in follicular growth during fetal life.

(b) Prepuberty

In primates, follicular growth, which has been begun during fetal life, continues throughout childhood (van Wagenen and Simpson, 1965; Peters *et al.*, 1976). All the follicles beginning to grow before puberty are doomed to degeneration. Follicles undergo atresia at any stage of their development, but the percentage of atretic follicles increases as follicular development advances (Vermande-van Eck, 1956; Koering, 1969; Himelstein-Braw *et al.*, 1976).

In other mammalian species follicular growth begins in prepubertal life. In fact, in rodents, the number of follicles starting to grow in the immature is much higher than their number in the mature animal. Follicles reach the antral stage and premature ovulation can be induced by exogenous gonadotrophins.

It seems that follicular development in prepubertal animals is related to the rise in basal levels of gonadotrophins in the blood. Administration of gonadotrophin antiserum to newborn mice did not prevent the initiation of follicular growth (Eshkol and Lunenfeld, 1972; Nakano *et al.*, 1975) but such treatment for 2 weeks resulted in poorly organized granulosa, ill-defined basal membrane and deficient development of the thecal layers and of follicular vasculature. Furthermore, normal follicular development could be restored by substitution with exogenous gonadotrophins. While follicle-stimulating hormone (FSH) restored normal preantral follicle formation, both FSH and luteinizing hormone (LH) were needed for the development of antral follicles (Eshkol *et al.*, 1970; Purandare *et al.*, 1976). These data seem to indicate that gonadotrophins affect the ovary even when the number of gonadotrophin receptors in granulosa cells is very low (Presl *et al.*, 1972, 1974) and before ovarian responsiveness to LH in terms of cAMP formation is established (Lamprecht *et al.*, 1973; Kolena, 1976).

1.6.3 DYNAMICS OF FOLLICULAR GROWTH IN MATURE MAMMALS

The dynamics of follicular growth in rodents has been examined by radiolabelling of granulosa cells (T. Pedersen, 1970; Chiras and Greenwald, 1977; Hage *et al.*, 1978). The kinetic aspects of follicular development in the rat have been discussed recently (Hirshfield and Schmidt, 1987). In the mature female, the growth-initiation rate is constant; in the mouse about 20 follicles begin to grow every day (T. Pedersen, 1972). Follicular growth is continuous and there is no evidence for arrest during growth (Peters and McNatty, 1980). Likewise, the rate of growth of human dominant follicles did not change under hormonal stimulation (Kerin *et al.*, 1981; Jacobs *et al.*, 1985; Templeton *et al.*, 1986; Baird, 1987). In the mouse it takes 19 days (i.e. four to five cycles) for a follicle that begins to grow to reach ovulation (T. Pedersen, 1972). In the sheep (Turnbull *et al.*, 1977; Dufour *et al.*, 1979; Cahill and Mauléon, 1980) and the human (Gougeon, 1982) about 6 months are needed for follicular growth. Most of this period is preantral development. It takes about 24 days for a sheep follicle to reach ovulation from the first appearance of the antrum (Turnbull *et al.*, 1977; Cahill and Mauléon, 1980) and about 60 days in the human (Gougeon, 1982, 1986).

1.6.4 GENERAL FEATURES OF FOLLICULAR GROWTH

The primordial follicle entering the growth phase undergoes two major changes: trans-

formation of the granulosa cells from flattened to cuboidal shape and increase in oocyte volume (Lintern-Moore and Moore, 1979). Further development includes mitotic proliferation of granulosa cells and their transformation into multilayered membrane granulosa. The earliest stages of thecal differentiation and their timing are obscure (Hirshfield, 1988).

In all species examined, follicular growth includes two phases. During the first phase there is a proportional increase in oocyte and follicular volume, while during the second one the oocyte increases only slightly, but the follicle continues marked growth, primarily due to accumulation of follicular fluid (Brambell, 1956). In most mammalian species, the final size of preovulatory follicles is related to body size (see Harrison and Weir, 1977).

The factors involved in initiation of follicular growth are obscure. Recruitment of follicles from the non-growing pool may be in a random fashion or orderly, i.e. oocytes entering the meiotic prophase first are recruited first into the pool of growing follicles and subsequently mature earlier. This latter view is referred to as the 'production line hypothesis' and was put forward by Henderson and Edwards (1968) on the basis of reduced chiasma frequency and parallel increase in chromosomal univalents in mouse oocytes. Data supporting the 'production line hypothesis' were obtained by labelling oocytes with tritiated thymidine *in vitro* and examination of the maturing oocytes after transplanting the ovaries *in vivo* (Polani and Crolla, 1981, 1989). However, the available data are not sufficient to prove any of these hypotheses. The rate of initiation of follicular growth is determined genetically and varies with the age of the female. Thus, it is highest in the young animal and is reduced with the approach of puberty. Once follicular growth is initiated, it continues until the follicle undergoes atresia or ovulates. Only a few of the follicles starting to grow reach ovulation and the vast majority degenerates. Follicular growth is associated with an increase in the proportion of atretic follicles (Pincus and Enzmann, 1937; Vermande-van Eck, 1956; Himelstein-Braw *et al.*, 1976). Various aspects of follicular atresia have been reviewed (Ingram, 1962; Weir and Rowlands, 1977; Byskov, 1978b; Peters and McNatty, 1980; Tsafriri and Braw, 1984; Hirshfield and Schmidt, 1987) and will not be dealt with.

Follicular growth entails a multitude of morphological and physiological changes in all follicular compartments. A brief account of some of these follows.

(a) The formation of zona pellucida

In growing follicles (type 3a, Figure 1.4) a homogeneous material forming pocket-like partitions between the oocyte and adjacent granulosa cells is deposited. These patches coalesce to form a microscopically continuous sheath around the oocyte, the zona pellucida. It is freely permeable to proteins of high molecular weight such as ferritin (Hastings *et al.*, 1972) and immunoglobulin M (Sellens and Jenkinson, 1975).

Scanning electron microscopy reveals that the surface of zona pellucida of the rat (Phillips *et al.*, 1978), the mouse and the hamster (Phillips and Shalgi, 1980) is characterized by numerous fenestrations rendering it of a sponge-like appearance. In transmission electron microscopy the zona appears as a homogeneous layer (Baca and Zamboni, 1967; Kang, 1974; Zamboni, 1974). Staining with ruthenium red (an electron-dense stain with high affinity for acidic mucopolysaccharides) differentiates between the outer and the inner layer of zona pellucida of mouse (Baranska *et al.*, 1975), pig (Hedrick and Fry, 1980) and rat (Wolgemuth and Gavin, 1981) oocytes.

The biology and biochemistry of the zona pellucida were recently reviewed by P.M. Wassarman *et al.* (1985) and Wassarman (1988). Studies in the mouse identified by electrophoresis three glycoproteins, desig-

nated ZP1 (≃ 120 kDa), ZP2 (≃ 120 kDa) and ZP3 (≃ 80 kDa). These account for virtually all the mass of the mouse zona pellucida (Bleil and Wassarman, 1980a). Competitive sperm binding assays demonstrated that ZP3, but not ZP1 and ZP2, possesses sperm receptor activity (Bleil and Wassarman, 1980b, 1983). Likewise, three major glycoproteins were identified in the zona pellucida of pig and rabbit oocytes (Dunbar *et al.*, 1981).

The cellular origin of the zona pellucida has been controversial (see Tsafriri, 1978). Recently, the synthesis of all three zona proteins by mouse denuded oocytes was demonstrated *in vitro* while ovectomized follicles (i.e. follicles from which the oocytes were removed) did not produce any zona proteins (Bleil and Wassarman, 1980c; Greve *et al.*, 1982; Shimzie *et al.*, 1983). By immunocytochemical procedures zona pellucida antigens were localized in cumulus cells of the hamster and the human (Bousquet *et al.*, 1981) and the rabbit (Wolgemuth *et al.*, 1984). It is not clear, however, whether this localization is indicative of cumulus cell synthesis of zona proteins or is a secondary result of endocytosis or phagocytosis of zona pellucida material. In fact, the immunocytochemical staining of cumulus cells was limited to the innermost layers of cumulus cells, appeared appreciably later than in the oocyte or the extracellular matrix and diminished once the oocyte was fully grown (Wolgemuth *et al.*, 1984). These findings are compatible with secondary distribution of zona proteins in cumulus cells, probably associated with formation of cumulus–oocyte channels. Thus, the available data clearly suggest the oocyte as the major source of the proteins of the zona pellucida.

(b) Intercellular communication within the follicle

The ovary, from its early stages of differentiation, is characterized by the close association of cells which allows functional coupling by intercellular communication. Groups of oogonia and oocytes are connected by intercellular bridges and the associated cells undergo developmental changes synchronously. These intercellular bridges disappear during enclosure of the diplotene oocytes by granulosa cells at folliculogenesis.

Throughout follicular development intercellular communication between homologous cells (i.e. granulosa or theca cells) and heterologous cells (i.e. granulosa–cumulus cells and the oocyte) is maintained through gap functions. Gap junctions are specialized intercellular channels allowing ionic coupling and transfer of low molecular weight metabolites between adjacent cells (Hertzberg *et al.*, 1981; Loewenstein, 1981; Macdonald, 1985). The physiological role of gap junctions, with special emphasis on the ovary, was reviewed by Schultz (1985).

The first observed gap junctions in the ovary appear between rete ovarii cells (presumptive progenitors of granulosa cells) in sex cords of 13-day-old mouse embryos (Mitchell and Burghardt, 1986). These gap junctions persist as the cells differentiate into granulosa cells. Furthermore, the number of junctional contacts increased during fetal development between days 15 and 19. The first heterocellular gap junctions were detected between oocytes and presumptive granulosa cells as early as fetal day 17 (Mitchell and Burghardt, 1986).

Gap junctions between conjoining granulosa cells of primordial follicles were observed in mouse embryos (Mitchell and Burghardt, 1986) and neonatal rats (Fletcher, 1979). However, they increase in number and size during follicular development (Albertini and Anderson, 1974) to form an extensive network of communication throughout the membrana granulosa compartment of antral and preovulatory follicles (Bjersing and Cajander, 1974a; Albertini *et al.*, 1975; Amsterdam *et al.*, 1976; Anderson and Albertini, 1976). Gap junctions were observed also in conjoining theca internal cells,

but they do not appear to reach the number and proportion of those of the granulosa cell compartment (Amsterdam *et al.*, 1976; Fletcher, 1979).

Formation of the zona pellucida brings about the separation of the oocyte and follicle cells. Simultaneously, both the oocyte and the surrounding follicle cells facing the oocyte develop cytoplasmic projections. Those of the follicle cells at first are short and bulky, and later on become longer and narrower. In the larger processes, cytoplasmic organelles can be seen frequently. The oolemma develops short microvilli, and the number of oocyte microvilli increases with follicular growth (Anderson and Beams, 1960; Hope, 1965; Baca and Zamboni, 1967; Hertig and Adams, 1967; Albertini and Anderson, 1974; Zamboni, 1974, 1976; Anderson and Albertini, 1976). Simultaneous appearance of micropapillae on the oolemma was described (H. Pedersen and Seidel, 1972; Zamboni, 1974, 1976). These appear as hemispherical, lens-shaped thickenings of oocyte membrane located mainly on the microvilli. It was suggested that the micropapillae enhance the adhesion of the oocyte microvilli to the follicle cell processes (Zamboni, 1974). The dilated, bulbous extremities of follicle cells terminate at the oocyte surface. In primates, very deep penetration of those processes into the oocyte was described (Hope, 1965; Baca and Zamboni, 1967; Zamboni, 1974, 1976).

The junctions between the oocyte and granulosa cells were identified as gap junctions (Amsterdam *et al.*, 1976; Anderson and Albertini, 1976). Unlike the gap junctions between granulosa cells (Merk *et al.*, 1973; Albertini and Anderson, 1974), those between the oocyte and the follicle cells are extremely small in size (Amsterdam *et al.*, 1976; Anderson and Albertini, 1976).

The extensive network of gap junctions between follicular granulosa cells and between the cumulus granulosa cells and the oocytes provides ionic coupling and transfer of regulatory molecules between all cells enclosed by the basement membrane (Schultz, 1985). Thus, in spite of the heterogeny among granulosa cells in terms of morphology (Zoller and Weisz, 1978), gonadotrophin binding (Amsterdam *et al.*, 1975), biosynthetic (Zoller and Weisz, 1979) and mitotic activity (Hirshfield, 1986), the gap junctions allow physiological integration of follicular responses. The suggested role of gap junctions and intercellular communication in oocyte growth and resumption of ovum maturation will be described below.

(c) Formation of theca layer

The formation of follicular theca layer is probably the least studied and understood (Harrison and Weir, 1977; Bjersing, 1978; Peters and McNatty, 1980; Hirshfield, 1988). Presumptive theca cells begin to appear in follicles with a single layer of cuboidal granulosa cells (Fletcher, 1979). The theca cells are first indistinguishable from fibroblasts and become aligned concentrically around the basement membrane. As the follicle grows, the theca layer thickens, the cells differentiate and develop smooth endoplasmic reticulum, large Golgi complexes and lipid droplets (Gillim *et al.*, 1969). Later, at the time of antrum formation, the theca differentiates into theca externa and interna (Brambell, 1928). The endocrine and physiological aspects of theca cells were discussed in Erickson *et al.* (1985) and Zlotkin *et al.* (1986).

(d) Hormonal correlates of follicular growth

The hormonal factors involved in follicular development, changes in follicle cell responsiveness to gonadotrophins and steroidogenic activity have been reviewed (Richards 1978, 1980; diZerega and Hodgen, 1981; Baird, 1983; Goodman and Hodgen, 1983; Baird, 1987; Greenwald and Terranova, 1988).

Initiation of follicle growth is apparently

not dependent upon gonadotrophic stimulation but is related to an as yet unknown signal, probably of ovarian origin. Thus, gonadotrophin deprivation by hypophysectomy or administration of antisera does not prevent growth of preantral follicles, whereas it does prevent the development of antral follicles (Mauléon, 1969; Eshkol *et al.*, 1970; Nakano *et al.*, 1975; Purandare *et al.*, 1976). Nevertheless, in the absence of gonadotrophins, the organization of the membrana granulosa and the theca layer is not normal (Eshkol *et al.*, 1970; Eshkol and Lunenfeld, 1972).

In vitro studies with 1- or 4-day-old rat ovaries seem to indicate that the ovary acquires some degree of steroidogenic activity independently of gonadotrophins (Funkenstein *et al.*, 1980; Funkenstein and Nimrod, 1981). Further development of growing follicles is influenced by the steroids produced in the ovary. In general, oestrogens enhance granulosa cell proliferation in rodents, whereas excessive levels of androgens are inhibitory and promote atresia (Payne and Runser, 1959; Goldenberg *et al.*, 1972). Follicular steroidogenesis was reviewed by Gore-Langton and Armstrong (1988).

Theca and ovarian interstitial cells are the major source of androgens (sheep, Seamark *et al.*, 1974; hamster, Makris and Ryan, 1980; rat, Fortune and Armstrong, 1977; rabbit, Erickson and Ryan, 1976a). The main enzymes necessary for the conversion of pregnenolone to androstenedione, C_{21} steroid 17α-hydroxylase and 17–20-desmolase, are located in microsomes (Ball and Kadis, 1965; Inano *et al.*, 1970). Androstenedione, a biologically weak androgen, is an important precursor of the more potent testosterone.

Oestradiol and oestrone are the major oestrogens synthesized by the follicle. The aromatizing enzymes converting C_{19} steroid (androgen) to C_{18} steroid (oestrogen) are located in the microsomes (Areco and Ryan, 1967). It is generally accepted that theca and granulosa cells act synergistically in oestrogen production. The so-called 'two-cell theory' was first proposed by Falck (1959), on the basis of his *in vivo* observations in the rat, and later modified and documented by *in vitro* studies in the hamster (Makris and Ryan, 1975, 1980), rabbit (Erickson and Ryan, 1975, 1976a), rat (Fortune and Armstrong, 1977) and sheep (Baird, 1977; Moor, 1977). This theory asserts that the theca interna cells, under the influence of LH, produce androgens that are transported to granulosa cells where they are converted to oestrogen by aromatizing enzymes induced by FSH. Thus, granulosa cells are the only source of follicular oestrogen in these species. However, in primate and porcine follicles the theca interna has been shown to provide an additional minor source of oestrogen (human, Channing, 1969; McNatty *et al.*, 1979a; monkey, Channing and Coudert, 1976; Channing, 1980; Marut *et al.*, 1983; Vernon *et al.*, 1983; sow, Haney and Schomberg, 1981; Stoklosowa *et al.*, 1982) and granulosa cells can produce androgens (human, Channing, 1969; McNatty *et al.*, 1979a,b; sow: Stoklosowa *et al.*, 1982), indicating that steroidogenesis is not rigidly compartmentalized in all species and throughout follicular differentiation (reviewed by Ryan, 1979; Hillensjö, 1981). An additional aspect of granulosa–theca cell synergism was suggested to include transfer of C_{21} steroids from the granulosa to the theca (Lischinsky and Armstrong, 1983; Fortune, 1986), thus involving the granulosa cells in thecal androgen synthesis.

It has been shown that theca cells of preantral and antral follicles have receptors for LH and that granulosa cells of most follicles, including small preantral ones, have receptors for FSH. Only in large preovulatory follicles do the granulosa cells also have receptors for LH (Eshkol and Lunenfeld, 1972; Presl *et al.*, 1972; Channing and Kammerman, 1973; Zeleznik *et al.*, 1974; Kammerman and Ross, 1975; Nimrod *et al.*, 1976; Richards *et al.*,

1976). Hence the development of receptors to LH in the granulosa cells seems to have a decisive role in follicle development and will be reviewed briefly.

The use of hypophysectomized immature rats has demonstrated the role of oestrogen and FSH in the proliferation of granulosa cells. Both hormones stimulated granulosa cell divisions and differential and their action was additive, suggesting action through different intracellular mechanism (Richards, 1975; Rao *et al.*, 1977). Further, oestrogen alone did not increase granulosa cell gonadotrophin receptors, but oestrogen priming enhanced FSH stimulation of the development of FSH and LH receptors on granulosa cells (Zeleznik *et al.*, 1974; Louvet and Vaitukaitis, 1976; Richards *et al.*, 1976; Ireland and Richards, 1978). As already mentioned, in addition to its effect on the increase of granulosa cell LH receptors, FSH enhances granulosa cell aromatization of androgen to oestrogen (Armstrong *et al.*, 1979).

LH also plays an important role in early follicular development. For example, human chorionic gonadotrophin (hCG) enhanced the effects of FSH in oestrogen-primed hypophysectomized rats (Ireland and Richards, 1978); in pregnant rats in the presence of elevated basal FSH sustained increases in serum LH (but not FSH) supported the development of preovulatory follicles (Richards, 1980; Bogovich *et al.*, 1981). In intact immature rats, small sustained increases in serum LH (hCG) stimulated the maturation of small antral follicles, and this effect of LH was enhanced by elevated concentrations of serum progesterone (Richards and Bogovich, 1982). This requirement for LH appears to be related to its stimulation of thecal cell differentiation and increase in thecal androgen biosynthesis. Thus, the development of ovulatory follicles is dependent upon the delicately balanced interactions of FSH, LH and steroid hormones. Any inappropriate change in the levels of gonadotrophins or steroids at critical stages of follicular development may lead to follicular atresia. Follicular growth is initiated continuously and, once follicles are recruited from the resting pool, they grow sequentially until they either become atretic or ovulate (Peters *et al.*, 1975). It seems, therefore, that from the continuum of growing follicles only those reaching a critical stage of their development coincident with the appropriate changes in serum gonadotrophin and steroid levels will be rescued and reach ovulation rather than atresia. Perhaps the most clearly demonstrated case of such selection is the rescue of early antral follicles (200–400 μm in diameter) by the pro-oestrous surge of FSH in the rat (Hirshfield and Midgley, 1978a,b). Similarly, rescue of follicles from atresia by PMSG was observed in mice (Peters *et al.*, 1975) and rats (Braw and Tsafriri, 1980). Accordingly, administration of an antiserum to FSH to pro-oestrous hamsters did not affect ovulation on the following night, but delayed ovulation at the next cycle (Sheela Rani and Moudgal, 1977).

Similar factors seem to be involved in the regulation of growth and selection of follicles in other species such as sheep, rhesus monkey and human (Ross and Lipsett, 1978; diZerega and Hodgen, 1981; McNatty, 1981; Hodgen, 1982; Baird, 1983; Goodman and Hodgen, 1983; Webb and Gauld, 1985; Baird, 1987). In sheep and in primates the preovulatory follicle becomes dominant a few days after the regression of the corpus luteum. The dominant follicles are characterized by increased secretion of oestrogen, a higher number of granulosa cells, measurable FSH and reduced androgen levels in follicular fluid (McNatty *et al.*, 1976, 1979c,d; McNatty and Baird, 1978; diZerega and Hodgen, 1981). It seems, therefore, that establishment of follicular dominance in the sheep and primates is due to an increase in the ability of granulosa cells to aromatize androgen precursor synthesized by the theca and thereby secreting large amounts of oestradiol. The dominant follicle, in turn, affects the development of follicles lower in hierarchy

towards atresia by suppressing FSH secretion by oestradiol (diZerega and Hodgen, 1981; Zeleznik, 1981; Zeleznik *et al.*, 1985) and inhibin (Schwartz and Channing, 1977; Channing *et al.*, 1982, 1985; Findlay and Risbridger, 1987) or by direct action of paracrine factor(s) (diZerega *et al.*, 1983a,b; Goodman and Hodgen, 1983; Cahill *et al.*, 1985a,b; Tonetta and diZerega, 1986). Nevertheless, the role of oestrogen in regulating follicular growth locally in primates and the human has been questioned recently. Thus, it has been shown that ovarian follicular development could be induced in a woman with 17α-hydroxylase deficiency in spite of extremely low ovarian concentrations of androgen and oestrogen (Rabinovici *et al.*, 1988). Furthermore, except for the ovarian germinal epithelium, oestrogen receptors were not detected in any other ovarian structure (Hild-Petito *et al.*, 1988; Stouffer *et al.*, 1993).

In the sheep, ovulation occurs within 3 days after natural or induced luteal regression, while in the rhesus monkey and the human the equivalent period is 14–19 days (Goodman *et al.*, 1977; Baird and McNeilly, 1981; Nilsson *et al.*, 1982; Goodman and Hodgen, 1983; Baird *et al.*, 1984). This difference may be attributed to the fact that in the sheep development of antral follicles (4–6 mm in diameter) continues throughout the luteal phase (Turnbull *et al.*, 1977; Cahill *et al.*, 1979). By contrast, in the human luteal phase, all follicles with a diameter above 4–6 mm are atretic (McNatty *et al.*, 1983). It was suggested that this difference in the pattern of follicular development is related to the pattern of steroid secretion by the corpora lutea (Baird *et al.*, 1975). Thus, in the ewe, the corpus luteum secretes only progesterone, whereas in the primates the corpus luteum secretes both progesterone and oestrogen, which suppresses release of gonadotrophins and hence further follicular development. Details on the hypothalamic–hypophyseal gonadal axis during oestrous and menstrual cycles can be found in chapters by Rowlands and Weir, Spies and Chappel and Yen and Lein in Volume 1 of this series.

Luteal regression in the sheep is followed by a rise in serum levels of LH and oestradiol, but not FSH (Baird *et al.*, 1981; Baird, 1983). This is accompanied by a marked decline in the percentage of healthy antral follicles, and by 36 h after luteolysis, except for the preovulatory follicle(s) all the antral follicles are atretic (McNatty *et al.*, 1982). It seems, therefore, that in the sheep the selection of the ovulatory follicle occurs early in the follicular phase. Both LH and FSH seem to be present during the luteal phase in minimal concentrations required for development of large antral follicles. FSH may be considered as having a permissive role and the rise in LH secretion seems to play an essential role in follicle selection in the sheep (Baird *et al.*, 1981).

Follicular rescue may be achieved during the luteal phase in the rhesus monkey by administration of exogenous gonadotrophin (diZerega and Hodgen, 1980). On the basis of experience with gonadotrophin induction of ovulation in hypogonadotrophic women, Brown (1978) suggested that follicles are selected by threshold levels of FSH, above which final stages of follicular development can proceed to ovulation. The difference between a threshold and subminimal dose may be as small as 20%. According to this concept, the modest rise in FSH concentration in the early follicular phase is sufficient to sustain follicular development beyond 4 mm size in spite of the later decrease in FSH levels. This hypothesis was recently supported in the macaque monkey (Zeleznik and Kubik, 1986). Endogenous gonadotrophins were blocked in these monkeys by a gonadotrophin-releasing hormone (GnRH) antagonist and increased amounts of FSH were infused until the threshold of follicular development, as monitored by serum oestradiol, was reached. Once follicle selection had occurred, reduction of FSH levels was accompanied by progressive rise in oestradiol

levels and preovulatory follicle growth. Thus, the selected follicle seems to continue its development in spite of falling FSH levels, insufficient to support the growth of smaller follicles. Following enucleation of corpora lutea in the human there was a fall in plasma levels of progesterone and oestradiol (Nilsson *et al.*, 1982; Baird *et al.*, 1984). The rise in gonadotrophin levels was delayed by 24 h following surgery and it preceded the rise in plasma oestradiol levels (Baird *et al.*, 1984). Luteectomy in the rhesus monkey resulted in a small rise in FSH concentrations, which was considered by the authors to be insignificant (Goodman *et al.*, 1979). It is possible that this is a result of infrequent blood sampling. Furthermore, the finding that only a small 20–30% rise in FSH for a limited period is required in the human (Baird *et al.*, 1984) and the macaque (Zeleznik and Kubik, 1986) for follicular rescue supports the notion that a rise in FSH is involved in follicle selection in primates.

1.6.5 OOCYTE GROWTH AND DEVELOPMENT OF MEIOTIC COMPETENCE

As already discussed, oocyte and follicular growth are interrelated. The increased rate of follicular growth initiation during neonatal development in rodents allows detailed studies on oocyte growth and biosynthetic activity. Most of these studies were performed using mouse oocytes (Bachvarova, 1985; Siracusa *et al.*, 1985; Van Blerkom, 1985; Schultz, 1986). Here we shall review some of the salient findings of these studies and relate them to the ability of the oocyte to resume the meiotic process.

The oocyte, during its growth, increases in size from 18–20 μm (Figure 1.5).

This is accompanied by marked changes in oocyte ultrastructure. Thus, early stages of oocyte growth are characterized by few and short microvilli, extensive network of smooth endoplasmic reticulum, elongated and dumbbell-shaped mitochondria with laminar mitochondrial crests which are associated with the endoplasmic reticulum. The ribosomes are in clusters, the Golgi apparatus consists of flattened stacks of parallel lamellae, and there is a rapidly enlarging fibrillogranular nucleolus. During growth, the number of microvilli increases and they become longer. The smooth endoplasmic reticulum becomes vesicular and less abundant. The number and size of mitochondria is reduced, they assume round or oval shape and their cristae are columnar and later on arched or arranged concentrically. The number of free ribosomes is reduced and ordered lattices appear and their number increases. The Golgi apparatus progressively becomes more vacuolated and granular, often associated with lipid droplets, the number of multivesicular bodies increases and also the nucleolus increases in its size and density (Zamboni, 1974; P.M. Wassarman and Josefowicz, 1978).

(a) Oocyte growth and intercellular communication

The junctional communication between cumulus–granulosa cells plays an important nutritional role in the dictyate oocyte (Schultz, 1985). Thus the cumulus–granulosa cells provide the major route of entry into the oocyte for most metabolites, such as energy sources, and precursors of RNA, protein and phospholipid biosynthesis (Cross and Brinster, 1974; P.M. Wassarman and Letourneau, 1976; Heller and Schultz, 1980; Moor *et al.*, 1980a; Heller *et al.*, 1981). This has been established by uptake and pulse chase experiments comparing intact cumulus cell–oocyte complexes and denuded oocytes. Treatments known to disrupt gap junctions in other cell types result in reversible breakdown of communication between cumulus cells and the oocyte (Heller *et al.*, 1981). Furthermore, the extent of communication between cumulus and the oocyte is directly

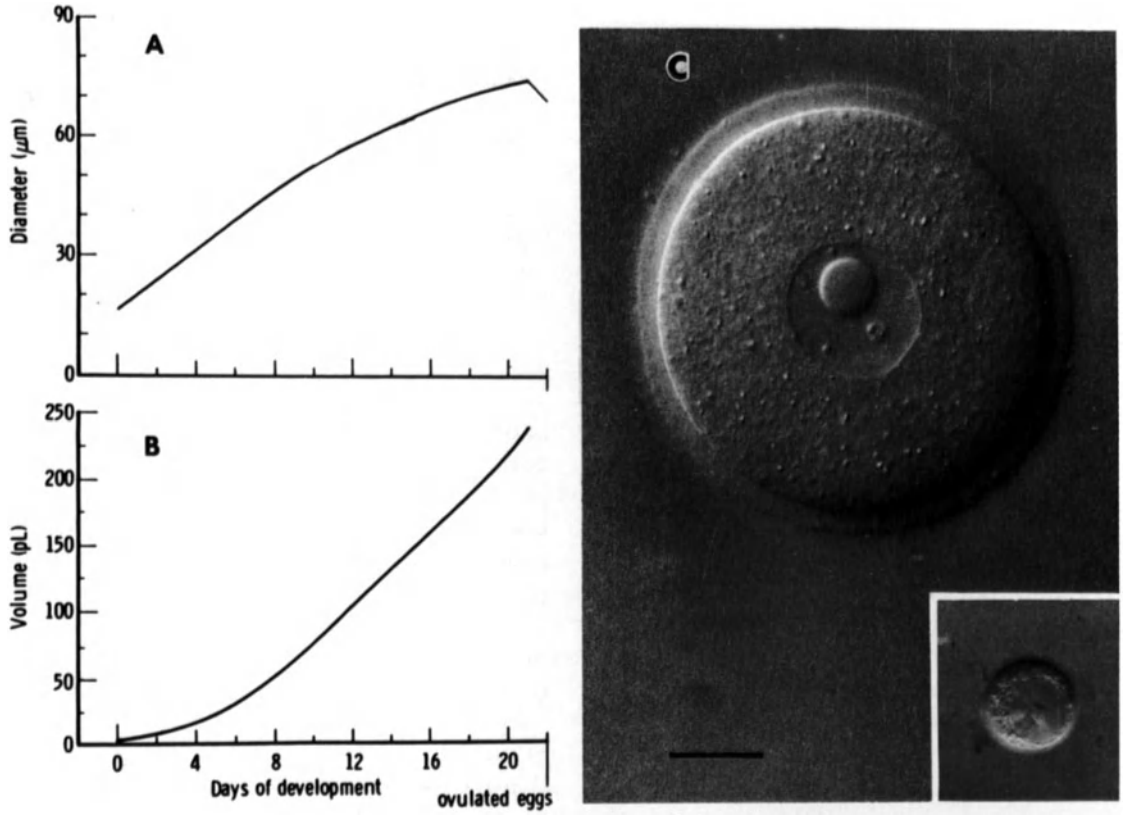

Figure 1.5 Growth of mouse oocytes. (A) The average diameter of freshly isolated oocytes from mice of increasing age. (B) Increase in oocyte volume with time, calculated from (A). (From Bachvarova, 1985.) (C) A fully grown and (inset) a quiescent mouse oocyte at the same magnification. The quiescent oocyte was isolated from 3-day-old mouse and the fully grown from an adult mouse. Differential interference contrast microscopy; bar=20 μm. (From Siracusa *et al.*, 1985.)

related to the number of attached cumulus cells (Brower and Schultz, 1982).

In explanted follicles, obtained by enzymatic digestion of ovaries, oocyte growth continues at a rate equal to that *in vivo*, in spite of the fact that many follicular cells migrate away and form a monolayer (Eppig, 1977, 1979). By contrast, denuded oocytes, free of attached cumulus cells, do not grow *in vitro*. Nevertheless, denuded oocytes cultured either directly on a monolayer of follicular cells or indirectly on ovarian cells covered by agar also grow *in vitro*, albeit at a considerably slower rate (Bachvarova *et al.*, 1980). Therefore, the presence of follicle cells is a prerequisite for oocyte growth, which can proceed at a lower rate even in the absence of junctional communication. The rate of oocyte growth is directly related to the extent of cumulus–oocyte communication (Brower and Schultz, 1982), and re-establishment of intercellular communication restores oocyte growth (Herlands and Schultz, 1984).

(b) Biosynthetic activity of growing oocytes

RNA synthesis

Early radioautographic studies demonstrated nuclear and nucleolar synthesis of RNA in

growing oocytes of the rat, mouse and monkey (Oakberg, 1968; Baker *et al.*, 1969). These were complemented later by assay of RNA polymerase activity in ovarian sections (Moore, 1978; Moore and Lintern-Moore, 1978) and *in vitro* incorporation studies into various RNA classes (Bachvarova, 1981; Brower *et al.*, 1981; Kaplan *et al.*, 1982; reviewed by Bachvarova, 1985; Schultz, 1986). The kinetics of RNA accumulation is biphasic: essentially all oocyte RNA is accumulated by the time they reach two-thirds of their final volume (Sternlicht and Schultz, 1981; Kaplan *et al.*, 1982).

The bulk of RNA synthesized by growing mouse oocytes is very stable, and about 80% of the radioactive RNA precursor detected 2 days after administration was retained in ovulated oocytes 10–20 days later (Bachvarova, 1974; Jahn *et al.*, 1976). Likewise, poly(A^-) RNA, predominantly rRNA and tRNA, has been estimated half-life of about 28 days and poly(A^+)RNA of about 10–14 days (Bachvarova, 1981; Brower *et al.*, 1981). Analysis of the distribution of newly synthesized cytoplasmic RNA revealed that about one-quarter of the newly synthesized mRNA and one-third of other rRNA appear in polysomes, thus suggesting that the bulk of ribosomal and messenger RNA is accumulated for later use (De Leon *et al.*, 1983). At the end of its growth period the mouse oocyte contains between 0.4 and 0.6 ng of total RNA (Olds *et al.*, 1973; Bachvarova, 1974; Sternlicht and Schultz, 1981; Kaplan *et al.*, 1982), i.e. about 200 times the amount of typical somatic cells. Of this, 10–15% is mRNA, 20–25% tRNA and 60–65% ribosomal RNA (P. Wassarman, 1983; Bachvarova, 1985). The transcription of several genes, the proto-oncogenes c-*mos* and c-*kit*, a transcription factor *oct-3*, two enzymes, t-PA and LDH (lactate dehydrogenase) and a sperm receptor *mZP3* during oogenesis has been recently reviewed (Eppig, 1991; P.M. Wassarman and Kinloch, 1992).

During oocyte growth in rodents, conspicuous fibrillar or lamellar structures accumulate in the cytoplasm (Weakley, 1967, 1968; Zamboni, 1970; Burkholder *et al.*, 1971; P.M. Wassarman and Josefowicz, 1978). Whether these structures, assuming lattice-like appearance in mouse oocytes, represent inactive ribosomes (Burkholder *et al.*, 1971) or yolk bodies (Szöllösi, 1972) remains to be determined (see Piko and Clegg, 1982; Bachvarova, 1985; Schultz, 1986).

Protein synthesis

During its growth phase the oocyte undergoes a 100-fold increase in its volume. Total protein content of the oocyte increases linearly with the increase in oocyte volume reaching 28–30 ng in the fully grown oocyte (Brinster, 1967; Schultz and Wassarman, 1977a). The absolute rate of protein synthesis during oocyte growth increases from 1.1 pg/h in non-growing oocytes to 45–48 pg/h in the fully grown oocyte (Canipari *et al.*, 1979; Shultz *et al.*, 1979a). Calculations based on this measured absolute rate of protein synthesis in denuded oocytes led Schultz *et al.* (1979b) to conclude that the oocyte could synthesize only half of its protein. However, these measurements may underestimate the rate of protein synthesis (Canipari *et al.*, 1979) and, furthermore, uptake of animo acids and protein synthesis in denuded oocytes is reduced as compared with follicle-enclosed oocytes (Crosby *et al.*, 1981; Salustri and Martinozzi, 1983). Bachvarova (1985), therefore proposed that the growing oocyte could synthesize all of its protein. In view of possible experimental errors, it is very difficult to conclude on the basis of these calculations whether part of oocyte proteins is produced elsewhere. In any case, uptake of serum proteins by the oocyte has been obtained *in vivo* (Glass, 1971) and evidence for active pinocytosis has been observed by electron microscopy (Zamboni, 1974).

The available information on synthesis of specific proteins during oocyte growth is lim-

ited to a few abundant proteins or enzymes that can be easily assayed (see Schultz, 1986).

Enzyme activity of both glucose-6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) increases during mouse oocyte growth. Only the specific activity of LDH increases with growth while that of G6PD remains essentially constant during growth (Mangia and Epstein, 1975; Mangia *et al.*, 1976; Casico and Wassarman, 1982). Likewise, mouse oocytes contain the brain isozyme of creatine kinase (up to 1.4% of total protein) and its activity increases during oocyte growth (Iyengar *et al.*, 1983). The activity of these enzymes may be related to oocyte metabolism. Thus, G6PD is probably needed for generation of NADPH, LDH in energy metabolism and creatine kinase for the maintenance of a high ATP/ADP ratio which is essential for biosynthetic activity of the oocyte during the period it cannot utilize glucose as an energy source (Biggers *et al.*, 1967; Donahue and Stern, 1968; Biggers, 1971, 1972).

The synthesis of tubulin (Schultz *et al.*, 1979a,b), actin (Kaplan *et al.*, 1982), histone H4 (P.M. Wassarman and Mrozak, 1981), ribosomal proteins (La Marca and Wassarman, 1979) and mitochondrial proteins (Mangia and Canipari, 1977; Cascio and Wassarman, 1981) has been examined in growing mouse oocytes. The mechanisms regulating the translation of c-*mos*, c-*kit*, *mZP3*, t-PA and LDH during mouse egg development were reviewed recently (P.M. Wassarman and Kinloch, 1992).

(c) The development of meiotic competence

The pioneering observation of Pincus and Enzmann (1935) that rabbit oocytes removed from their follicles resume the meiotic process *in vitro* has been confirmed and was extended to many other mammalian species (Donahue, 1972; Schuetz, 1974; Tsafriri, 1978). However, more recent studies have revealed that the ability of such explanted oocytes to undergo maturation *in vitro* (i.e. meiotic competence) is attained only at an advanced stage of follicle and oocyte growth; oocytes collected from immature mice prior to day 15 post partum failed to resume maturation when cultured. On the other hand, oocytes harvested from young mice 15 days post partum and older matured *in vitro* and the incidence of oocytes achieving maturation increased with the age of the animal at explantation (Szybek, 1972; Sorensen and Wassarman, 1976). Meiotic competence is acquired between days 15 and 21 of age in mice (Szybek, 1972; Sorensen and Wassarman, 1976), at 23 days in hamsters (Iwamatsu and Yanagimachi, 1975) and between days 20 and 26 in rats (Bar-Ami and Tsafriri, 1981). In mouse littermates the mean diameter of competent oocytes was larger than that of incompetent oocytes (Sorensen and Wassarman, 1976). In the pool of oocytes explanted from rats between 15 and 31 days post partum there was a high correlation ($r = 0.98$) between the ability of oocytes to resume meiosis in culture and oocyte diameter (Bar-Ami and Tsafriri, 1981). However, in the rat the oocyte diameter is probably not the only determinant of meiotic competence, as the average diameter of the few competent oocytes on day 20 (61.8 ± 1.2 μm) was below the average diameter of incompetent oocytes on day 26 (76.5 ± 0.8 μm; $P < 0.001$). Likewise, dissociation between acquisition of meiotic competence and oocyte growth was observed in mouse oocytes cultured *in vitro*. Incompetent denuded oocytes attained competence when cultured with fibroblast monolayers, although growth was arrested (Canipari *et al.*, 1984).

It was suggested that meiotic competence is reached at the time of antrum formation (Brambell, 1928; Pincus and Enzmann, 1937). Erickson and Sorensen (1974) demonstrated in mice that only oocytes explanted from antral, but not from preantral, follicles of adult animals resumed meiosis in culture. In histological sections of ovaries obtained from

20-, 24- and 26-day-old rats, there was an increase in the percentage of antral follicles from 14.3 to 38.8%, *pari passu* with the acquisition of meiotic competence (Bar-Ami and Tsafriri, 1981). In contrast to rodents, in the pig, only 15% of the oocytes explanted from small antral follicles (approximately 1–2 mm in diameter) matured in culture, whereas 80% of oocytes explanted from large follicles (6–12 mm) were meiotically competent (Tsafriri and Channing, 1975a). In a more detailed study, in which follicles were first dissected and measured accurately, it was found that more than 80% of oocytes from follicles 0.3–0.7 mm in diameter remained immature, while 66, 94.3 and 100% of oocytes from follicles 0.8–1.6, 1.7–2.2 and 3.5 mm in diameter, respectively, resumed maturation within 24 h of culture (Motlik *et al.*, 1984a).

Studies with oocytes of various species, such as those from immature rabbits (Thibault, 1972), mice (Sorensen and Wassarman, 1976), rats (Bar-Ami and Tsafriri, 1981) and small antral follicles of the pig (Tsafriri and Channing, 1975a; Motlik *et al.*, 1984a), showed high incidence of incomplete oocyte maturation (mainly arrest at metaphase I). In human oocytes, explanted from large follicles (9–15 mm), a higher proportion of oocytes resuming maturation *in vitro* extruded the polar body, as compared with those from smaller follicles (< 9 mm) (Tsuji *et al.*, 1985). Likewise, all oocytes resuming meiosis in culture with fibroblast monolayer were arrested at metaphase I of meiosis (Canipari *et al.*, 1984). Apparently, the capability of oocytes to mature is acquired gradually; the ability to resume meiosis and to undergo germinal vesicle breakdown (GVB) is attained earlier than the ability to reach metaphase II.

(d) Cytoplasmic factors and meiotic competence

It was demonstrated that fusion of incompetent mouse oocytes with oocytes that have resumed meiosis results in meiotic changes in the incompetent nucleus (Balakier, 1978; Tarowski and Balakier, 1980). This cytoplasmic activity is ascribed to a maturation promoting factor (MPF), involved in G_2 to M transition in both mitotic and meiotic cells (Masui and Clarke, 1979; Gerhart *et al.*, 1984). Currently, a more appropriate name for the MPF abbreviation is used: M-phase promoting factor (Gerhart *et al.*, 1984; H.H. Hashimoto and Kishimoto, 1988). MPF activity in cytoplasm of mature mouse oocytes was confirmed by injection of cytoplasm into *Asterina pectinifera* (Kishimito *et al.*, 1984) or *Xenopus laevis* (Sorensen *et al.*, 1985) oocytes. In both cases mouse cytoplasm induced resumption of meiosis in the absence of the hormonal stimulator of meiosis, progesterone in *Xenopus* and 1-methyladenine in *Asterina*.

Recently, evidence for the presence of an ooplasmic meiosis-inhibiting activity (MIA) has been obtained. Thus, fusion of growing incompetent oocytes with competent ones prevented the resumption of meiosis by the competent nucleus. Fusion of incompetent mouse or porcine oocytes prevented maturation of oocytes of the same species, and growing porcine oocytes prevented the maturation of mouse competent oocytes (Fulka, 1985; Fulka *et al.*, 1985). When the competent porcine oocytes were preincubated for 12–14 h, meiosis was resumed by nuclei after fusion (Fulka *et al.*, 1985). The precise molecular mechanisms involved in acquisition of meiotic competence remain to be elucidated. The recent purification of MPF (Gautier *et al.*, 1988; Lohka *et al.*, 1988), the demonstration of its protein kinase activity and the role of phosphorylation/dephosphorylation cascade in the regulation of mitotic cell cycle and of meiosis (Gautier *et al.*, 1989; Murray and Kirschner, 1989; Murray *et al.*, 1989) offer plausible mechanisms which may be involved in the acquisition of meiotic competence in mammalian oocytes. Indeed, microinjection of an inhibitor of cAMP-dependent protein kinase (PKI) failed to induce GVB in incompetent oocytes, but did so

readily in fully grown ones (Bornslaeger *et al.*, 1988).

(e) Does the development of meiotic competence depend on hormonal stimuli?

The development of follicular antrum appears to be dependent upon gonadotrophic stimulation (Richards, 1980). The correlation between antrum formation and acquisition of meiotic competence in rodents may suggest that the latter is also dependent upon gonadotrophins. Hypophysectomy of immature rats, prior to the acquisition of meiotic competence, was performed in order to test this hypothesis (Bar-Ami and Tsafriri, 1981). Hypophysectomy of rats on day 20 of age affected only slightly the ability of oocytes to resume maturation when explanted on day 26 (the percentage of oocytes undergoing GVB was reduced by hypophysectomy by 9.9 ± 5.8%). On the other hand, hypophysectomy on day 15 of life reduced the percentage of competent oocytes on day 26 by 58.1 ± 4.9% and their absolute number (3.9 ± 0.7 per rat in hypophysectomized rats as compared with 46.3 ± 3.9 competent oocytes per rat in intact rats of the same age) (Figure 1.6; Bar-Ami and Tsafriri, 1981).

Administration of PMSG (3 i.u.) on days 25, 27 and 29 of age resulted in a gradual increase in the percentage of competent oocytes during the 6-day treatment, from 41.9 ± 1.5 on day 27 to 94.5 ±1.5 on day 31. PMSG also increased the number of competent oocytes (46.8 ± 0.5 oocytes per rat as compared with 3.9 ± 0.7 per rat in hypophysectomized rats which were not treated with PMSG). The action of PMSG on meiotic competence appears to be due to its FSH-like activity: only FSH (NIAMD-rat-FSH) but not LH (NIAMD, purified-ovine-LH, Batch 21), was able to elicit this response when injected on days 25–28 to rats hypophysectomized on day 15 (Bar-Ami *et al.*, 1983).

The action of FSH (or PMSG) to increase the number and the percentage of competent oocytes is, at least partly, mediated by ovarian oestrogen production. Inhibitors of steroidogenesis such as aminoglutethimide (of cholesterol 20α-hydroxylase; Kahnt and Neher, 1966) and of aromatase (Thompson and Siiteri, 1974), 17β-formamidoandrost-4-en-3-one (of 17 – 20 lyase; Arth *et al.*, 1971) or of aromatase such as 1,4,6-androstatriene-3,17-dione (Schwarzel *et al.*, 1973) or 4-hydroxy-androstene-3,17-dione (Brodie *et al.*, 1977) prevented the effect of FSH on the

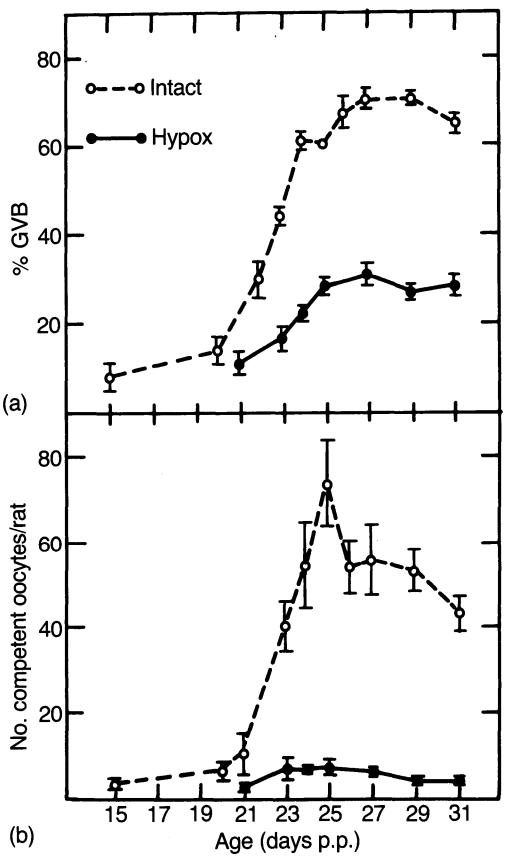

Figure 1.6 The acquisition of meiotic competence with age in intact and rats hypophysectomized (Hypox) on day 15 post partum (p.p.). Competence was tested by culture of isolated ova for 6 h. Mean and SEM are indicated. (Data from Bar-Ami and Tsafriri, 1981.)

acquisition of meiotic competence. But when oestradiol was administered together with these inhibitors of steroidogenesis, the effect of FSH on the development of meiotic competence was not disturbed (Bar-Ami *et al.*, 1983). The hormonal treatments (PMSG or oestradiol) induced meiotic competence provided that the treatment started after day 20 of age. Hormonal treatments at an earlier age were not effective in inducing meiotic competence (Bar-Ami and Tsafriri, 1986). Hypophysectomy of rats on day 30 (Erickson and Ryan, 1976b) or of mice on days 23–27 (Smith and Tenney, 1979) did not cause any consistent change in the percentage of oocytes undergoing GVB in culture, but it did reduce the number of oocytes arrested before reaching the second metaphase and giving off the polar body. Furthermore, the effect of hypophysectomy on the completion of meiosis in mouse oocytes was partially overcome by administration of PMSG or oestrogen. It appears then that hypophyseal gonadotrophins are involved also in the development of the capacity to complete the first meiotic division. Collectively, these observations support the notion that the development of meiotic competence is dependent upon hormonal stimulation.

By contrast, other observations support another view, that development of meiotic competence is a programmed event independent of hormonal stimulation. Thus, even in hypophysectomized rats there is no absolute depletion of competent oocytes (Bar-Ami and Tsafriri, 1981, 1986). Furthermore, a small proportion of incompetent oocytes cultured *in vitro* within their cumulus cells (Eppig, 1977), or on monolayers of follicle cells (Bachvarova *et al.*, 1980) or even fibroblasts (Canipari *et al.*, 1984), attained meiotic competence. Finally, grown oocytes (diameter ± 40 μm) from immature hypogonadal (hpg) mice that are deficient in hypothalamic gonadotrophin-releasing hormone and have thread-like uteri and very small ovaries (Cattanach *et al.*, 1977) resume meiosis to the same extent as their littermate controls (Schroeder and Eppig, 1989). It should be noted that in all cases of apparently autonomous acquisition of meiotic competence only a small number of oocytes became competent. Treatment of hpg mice with gonadotrophin promotes the development of meiotic competence by virtually all the oocytes (Schroeder and Eppig, 1989). Therefore, the normal extent of meiotic competence appears to require gonadotrophic support.

In conclusion, it seems that the development of meiotic competence includes both programmed events which cannot be triggered precociously by hormonal treatments (Bar-Ami and Tsafriri, 1986) and changes depending on endocrine stimulation. The latter are probably of a permissive nature and are required for the full extent of normal development of meiotic competence. As there is no conclusive evidence for gonadotrophin or oestrogen receptors in mammalian oocytes, the action of these hormones on meiotic competence is probably exerted indirectly through their action on the somatic compartment of the follicle.

1.7 OVULATORY CHANGES

The ovulatory response is a composite one, encompassing a multitude of biochemical changes, primarily in the mature follicle(s), but also in other ovarian compartments. In biological terms, at least three distinct components of ovulation can be discerned: the reinitiation of the meiotic process in the oocyte; a shift from follicular oestrogen to progesterone production with early luteinization; and finally, the rupture of follicular wall with delivery of a fertilizable oocyte. As ovarian steroidogenesis is the subject within a forthcoming volume in this series we shall follow here the life history of the mammalian ovum and discuss the resumption of meiosis and the release of the mature ovum with the rupture of the follicle.

1.7.1 RESUMPTION OF OVUM MATURATION

As already described (section 1.4), the meiotic process is arrested in mammals at the diplotene stage. Meiosis is reinitiated in healthy follicles only a few hours prior to follicular rupture at ovulation. Reinitiation of meiosis includes (McGaughey, 1983): (i) chromatin condensation in the germinal vesicle; (ii) disintegration of the nuclear membrane, germinal vesicle breakdown (GVB); (iii) formation of the first meiotic spindle; (iv) completion of the first meiotic division and abstriction of the first polar body; and (v) formation of the second meiotic spindle (Figures 1.1 and 1.7).

In most mammals, at follicular rupture, a secondary oocyte arrested at the metaphase of the second meiotic division is released. We shall refer to all these changes as **oocyte maturation**. Since GVB is the first prominent change readily observed in living oocytes of many species by interference or phase-contrast microscopy, this is taken as the criterion for resumption of the meiotic process (Figure 1.7). Therefore, the use of the terms **resumption of oocyte maturation** or **of meiosis** is preferable when only GVB was observed. While the presence of intact germinal vesicle is a valid criterion for confirming that the meiotic process is still arrested, only the abstriction of the first polar body and the presence of second meiotic spindle confirm the completion of oocyte maturation. Nevertheless, these nuclear changes do not necessarily assure physiological maturation of the ovum and its ability for normal fertilization and embryonic development (Moor *et al.*, 1980b; Osborn *et al.*, 1986). Nuclear maturation is accompanied by changes in the structure and organization of the cytoplasm (Zamboni, 1970; Van Blerkom and Motta, 1979; Van Blerkom and Runner, 1984) and the plasma membrane (Johnson *et al.*, 1975; Nicosia *et al.*, 1977; Maro *et al.*, 1986). Changes at the molecular level will be addressed in later sections.

Using nocodazole, a potent inhibitor of microtubule polymerization and transfer of bivalent chromosomes, Van Blerkom and Bell (1986) demonstrated the role of chromosomes for cytoplasmic and plasma membrane differentiation in maturing mouse oocytes. Furthermore, the capacity of the cytoplasm and plasma membrane to differentiate in response to the presence of a chromosome is acquired before GVB.

(a) Gonadotrophins and resumption of oocyte maturation *in vivo*

Oocyte maturation, like other ovulatory processes, is triggered by pituitary gonadotrophins. Thus, administration of hCG induces meiosis in the mouse (Edwards and Gates, 1959) and the pig (Hunter and Polge, 1966). Blocking the preovulatory surge of gonadotrophins in the rat by Nembutal or similar drugs also prevents the resumption of meiosis (Ayalon *et al.*, 1972). Likewise, hypophysectomy (Vermeiden and Zeilmaker, 1974) or administration of an antiserum to the β-subunit of LH (Tsafriri *et al.*, 1976d) blocks oocyte maturation. Conversely, administration of exogenous LH to such Nembutal-treated rats results in GVB within 2.5–3.5 h (Vermeiden and Zeilmaker, 1974; Magnusson *et al.*, 1977).

Both LH and FSH preparations are capable of inducing ovulation (Harrington *et al.*, 1970; Nuti *et al.*, 1974; Schwartz *et al.*, 1975). Furthermore, the preovulatory surge of gonadotrophins includes FSH as well as LH. Therefore, it was of interest to test whether the FSH released during the surge can trigger ovulation by itself. Indeed, immunopurified (Tsafriri *et al.*, 1976a) or human recombinant FSH (Galway *et al.*, 1990) induced the ovulatory response. Nevertheless, administration of an antiserum to β-LH to rats on the afternoon of pro-oestrus prevented ovum maturation and ovulation (Tsafriri *et al.*, 1976a). Hence, it was concluded that in the rat the amount of FSH secreted is not sufficient to

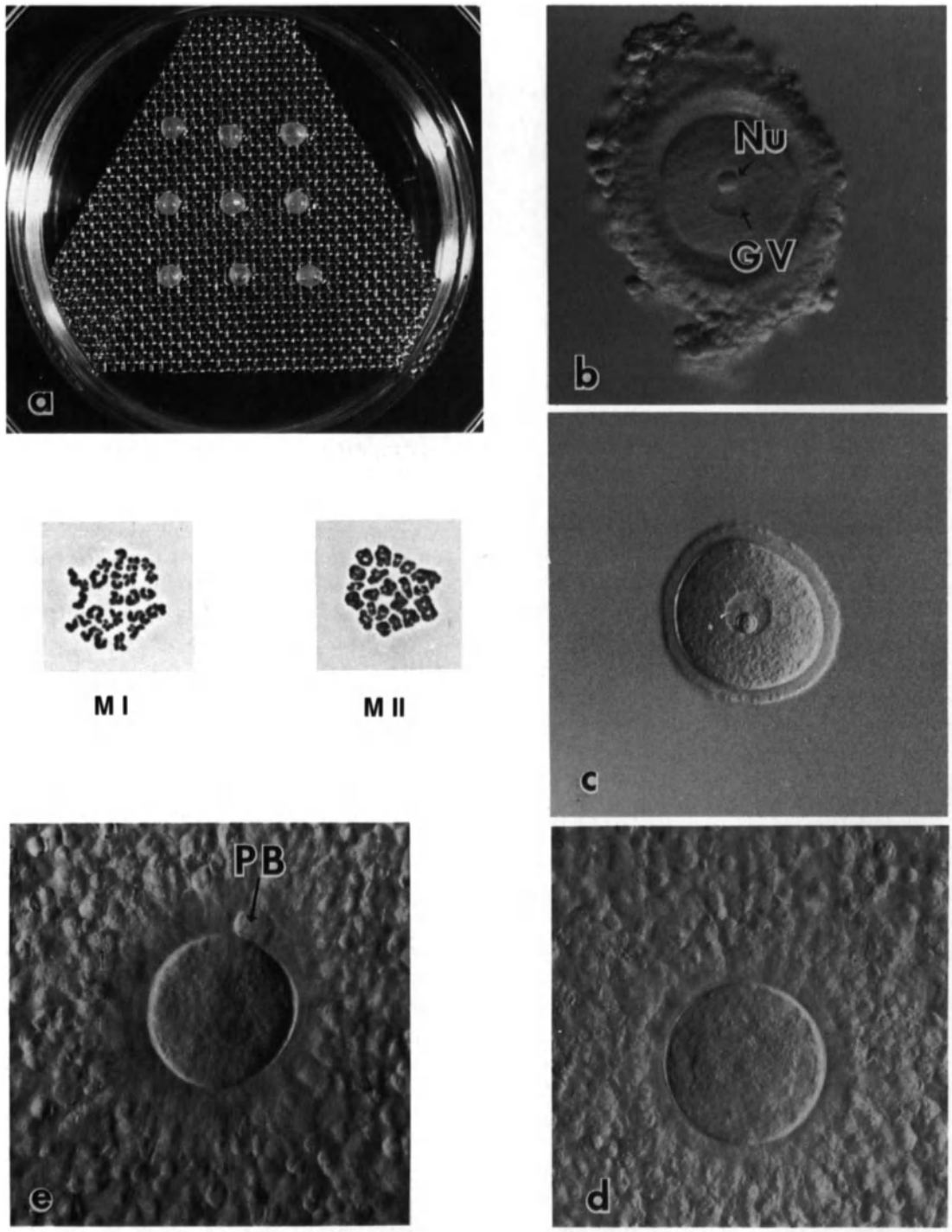

Figure 1.7 Oocyte maturation in the rat. (a) Explanted preovulatory follicles (b–e) Oocytes viewed with Nomarski interference optics; isolated oocyte with its intact cumulus mass (b). GV, germinal vesicle; Nu, nucleolus. (c) Denuded oocyte. (d) GVBD; M I (inset), bivalents in metaphase I. (e) Secondary oocyte. PB, polar body; M II (inset), chromosomes (dyads) seen at metaphase II. Chromosomes were prepared according to the method of Tarkowski (1966) and photographed under phase contrast. (From Tsafriri, 1985.)

induce ovulation and LH is the physiological trigger of ovulatory changes. The action of FSH on ovulation in these experiments must be regarded as merely a pharmacological effect.

In naturally ovulating mice, rats and pigs, oocyte maturation and follicular rupture at ovulation are separated only rarely, and only few immature oocytes can be detected in the fallopian tube. Hormonal stimulation of ovulation increases the number of immature ova released (Donahue, 1972). Precocious treatment of pigs by hCG resulted in ovulation of only immature ova (Hunter *et al.*, 1976).

(b) Oocyte maturation *in vitro*

Maturation of oocytes *in vivo* does not allow detailed examination of the mechanisms involved in the control of meiosis. Therefore, several *in vitro* model systems have been employed. These allow experimentation directed at identifying both the mechanisms involved in the maintenance of meiotic arrest in competent oocytes and those involved in the resumption of meiosis as a result of gonadotrophic stimulation. The simplest *in vitro* system employed for studying oocyte maturation was first established by Pincus and Enzmann (1935), showing that rabbit oocytes liberated from their follicles undergo maturation *in vitro* spontaneously without requiring hormonal stimulation. This phenomenon has been confirmed in all mammalian species examined (reviewed by Donahue, 1972). Isolated oocytes, denuded from their enclosing cumulus oophorus cells or within their cumulus complex, are widely employed for studying some physiological aspects of oocyte maturation (Figure 1.7b and c). This model, however, does not represent the physiological events, since oocyte maturation *in vivo* is dependent upon the release of hypophyseal gonadotrophin. It is possible that the removal of the oocyte from its follicular environment triggers the resumption of meiosis by a specific or unspecific signal.

Explantation of preovulatory follicles (Figure 1.7a) provides an *in vitro* model in which the normal association between various follicular cell types and compartments is maintained. In this model resumption of meiosis is dependent on hormonal stimulation (Tsafriri *et al.*, 1972a; Neal and Baker, 1973; Thibault and Gerard, 1973; Moor and Trounson, 1977; Thibault, 1977; Meinecke and Meinecke-Tillman, 1981). This model permitted the analysis of several mechanisms underlying the hormonal induction of meiosis: the clarification of the role of gonadotrophins, steroids, prostaglandins, cyclic AMP, macromolecular synthesis and follicular energy metabolism (Tsafriri, 1978). However, being a multicompartmental model it does not allow the localization of the primary site of gonadotrophin action in the induction of meiosis or the elucidation of the cascade of events involved in this response.

The contrasting behaviour of the isolated oocyte and of follicle-enclosed oocyte led to the hypothesis that the maturation of mammalian oocyte is normally prevented by an inhibitory action exerted by the follicular cells and that LH induces the resumption of meiosis by removing this restraint rather than by providing a positive stimulus. In order to test this hypothesis a third model was developed: co-culture of isolated oocytes with various follicular components (Foote and Thibault, 1969; Tsafriri and Channing, 1975b; Leibfried and First, 1980a). This approach, however, may be difficult to interpret as the oocyte as well as follicle cells are removed from their natural environment, which may result in changes relevant to meiosis in both compartments. Furthermore, the normal association between the oocyte and the follicle cells, as well as between follicle cells themselves, is disrupted and the system is still too complex to allow localization of specific cellular responses. Nevertheless, the isolated oocytes in culture, whether alone or with other follicular constituents, provide an indispensable approach for examination of mechanisms in-

volved in the maintenance of meiotic arrest. Recently, hormonal stimulation of granulosa cell–oocyte co-cultures has been proven as a convenient method for obtaining mature and developmentally competent ovine (Crosby *et al.*, 1981; Staigmiller and Moor, 1984), bovine (Hensleigh and Hunter, 1985; Critser *et al.*, 1986; Gordon and Lu, 1990) and porcine (Mattioli *et al.*, 1988a,b) ova (reviewed by Racowsky, 1991).

(c) Maturation of follicle-enclosed oocytes

Extensive studies on the maturation of follicle-enclosed oocytes were carried out in the rat. Similar results were obtained whether the preovulatory follicles were explanted from pro-oestrous mature cyclic rats (Tsafriri *et al.*, 1972a) or from immature PMSG-treated rats (Hillensjö, 1976). It was shown that oocytes explanted within their follicle on the day of pro-oestrus before 14:00 h, that is before the preovulatory surge of LH, remain in the dictyate stage throughout the 24 h culture period. By contrast, when the follicles were explanted after the endogenous surge of gonadotrophins, the oocytes matured in hormone-free medium.

Preovulatory follicles that are explanted prior to the surge of gonadotrophins can be used to test the meiosis-inducing action of hormones and other drugs. Meiosis is triggered in such rat follicles by LH, hCG, FSH or prostaglandin E_2 (PGE_2) (Tsafriri *et al.*, 1972a; Hillensjö, 1976). All these hormones induce a rise in follicular cyclic AMP (Lindner *et al.*, 1974), and their meiosis-inducing action is most probably mediated by this response. The recent demonstration that forskolin, a potent activator of adenylate cyclase, can by itself induce maturation of follicle-enclosed rat oocytes supports this notion (Dekel and Sherizly, 1983). The possible role of other follicular responses to LH in the mediation of its meiosis-inducing action was explored. Thus, inhibition of follicular steroidogenesis did not prevent the meiosis-inducing action of LH (Lieberman *et al.*, 1975). The role of steroids in the regulation of oocyte maturation will be discussed in detail. Inhibition of the stimulatory effect of LH on follicular lactate production did not prevent its action on resumption of meiosis (Tsafriri *et al.*, 1976b). By contrast, inhibition of protein synthesis by cycloheximide or puromycin abolished both resumption of meiosis and the enhancement of steroidogenesis (Lieberman *et al.*, 1976).

Resumption of meiosis was induced by gonadotrophins in follicle-enclosed oocytes of sheep (Hay and Moor, 1973; W.A. Miller and Jagiello, 1973; Moor and Trounson, 1977), cow (Thibault *et al.*, 1975a,b), pig (Gérard *et al.*, 1979; Meinecke and Meinecke-Tillman, 1981), monkey (Thibault *et al.*, 1976), rabbit (Thibault and Gérard, 1973) and hamster (Gwatkin and Andersen, 1976). Furthermore, in the rabbit (Thibault *et al.*, 1975a,b) and in the sheep (Moor and Trounson, 1977), the birth of normal young oocytes which had matured in follicles cultured in the presence of gonadotrophins was achieved.

Meiosis was also induced in follicle-enclosed rat oocytes by factors which do not enhance follicular cyclic AMP production. Thus, the hypothalamic gonadotrophin-releasing hormone (GnRH) or its agonistic analogue induced resumption of meiosis both *in vivo* (Ekholm *et al.*, 1981) and *in vitro* (Hillensjö and LeMaire, 1980). Whereas a GnRH antagonist abolished the meiosis-inducing action of GnRH or its analogue, it did not block this effect of LH (Ekholm *et al.*, 1982; Dekel *et al.*, 1983). It appears, therefore, that GnRH or a similar ovarian factor does not mediate the effect of LH on meiosis. GnRH induces follicular prostaglandin synthesis (M.R. Clark *et al.*, 1980), and $PGF_{2\alpha}$ was shown to induce resumption of meiosis (Tsafriri *et al.*, 1972a). However, indomethacin, a potent prostaglandin synthetase inhibitor, blocked the effect of GnRH analogue on ovulation but not on oocyte maturation (Ekholm *et al.*, 1982), suggesting that induction of meiosis by GnRH is not mediated by

follicular prostaglandin synthesis. The effect of GnRH is probably exerted via the activation of protein kinase C (Dekel *et al.*, 1989).

Other drugs induce resumption of meiosis in follicle enclosed oocytes: clomiphene citrate (0.01–0.5 nM) (Laufer *et al.*, 1982); tetrahydrocannabinol (100–200 μ), the major psychoactive ingredient of marihuana and cannabidiol (100–200 μ), one of its non-psychoactive constituents (Reich *et al.*, 1982). Likewise, epidermal growth factor (Dekel and Sherizly, 1985) and transforming growth factor alpha (Tsafriri *et al.*, 1989a) induce maturation of follicle-enclosed rat oocytes. The demonstration that activators of protein kinase C, such as phorbol ester, synthetic diacylglycerol and phospholipase C, also induce meiosis in follicle-enclosed oocytes (Aberdam and Dekel, 1985; Dekel *et al.*, 1989) suggests that a cyclic AMP-independent mechanism may also be involved in oocyte maturation. Nevertheless, the precise role of mechanisms involving protein kinase C in regulation of oocyte maturation remains to be elucidated (see Protein phosphorylation).

(d) Follicular control of oocyte maturation

Inhibitory effect of granulosa cells and follicular fluid

The spontaneous maturation of isolated oocytes in culture led Pincus and Enzmann (1935) to suggest that follicle cells in mammals 'supply to the ovum a substance or substances which directly inhibit nuclear maturation'. This suggestion was supported by the inhibition of the resumption of meiosis of porcine oocytes co-cultured with segments of follicle wall, but not with its theca layer (Foote and Thibault, 1969; Tsafriri and Channing, 1975b) or ovarian bursa (Leibfried and First, 1980a). Likewise, meiosis was inhibited in porcine oocytes transferred to host follicles (Meinecke and Meinecke-Tillmann, 1981; Fleming *et al.*, 1985) or planted onto extroverted follicles (Mattioli *et al.*, 1986) and cultured in hormone-free medium, and maturation of rat oocytes injected into porcine follicles was markedly delayed (Fleming *et al.*, 1985). Further, co-culture of porcine oocytes with porcine granulosa cells inhibited the spontaneous resumption of meiosis (Tsafriri and Channing, 1975b; Sato and Ishibashi, 1977). The inhibitory effect of granulosa cells depended upon their concentration in culture. Granulosa cells collected from small follicles inhibited meiosis more effectively than cells from medium or large follicles (Tsafriri and Channing, 1975b; Centola *et al.*, 1981). Similarly, when rat oocytes were added to rat granulosa cells previously cultured for 24 or 48 h, resumption of meiosis was suppressed and the degree of inhibition depended upon the number of granulosa cells in the culture. This inhibitory effect of rat granulosa cells was reversed by the addition of LH to the co-cultures (Tsafriri *et al.*, 1977; Tsafriri, 1979). Nuclear maturation of rabbit oocytes was significantly delayed when cultured with granulosa cells (Motlik and Fulka, 1981). Likewise, the spontaneous maturation of bovine oocyte was suppressed by co-culture with granulosa cells (Sirad and Bilodeau, 1990a,b).

Extracts of granulosa cells, as well as granulosa cell-conditioned medium, i.e. a medium in which granulosa cells were cultured previously, inhibited the resumption of meiosis (Tsafriri *et al.*, 1976c; Sato and Ishibashi, 1977; Centola *et al.*, 1981). Collectively, these results suggest that within the follicle meiosis is arrested by an oocyte maturation inhibitor (OMI) produced by granulosa cells. It was found that addition of FSH or prolactin to suspension cultures of porcine granulosa cells increases OMI activity in the medium, whereas testosterone or dihydrotestosterone reduces it (Channing *et al.*, 1982).

Follicular fluid (FF) from ovaries of rabbit, ovine, bovine, porcine, hamster and human origin exerted an inhibitory effect upon the spontaneous maturation of isolated oocytes

(Chang, 1955; Tsafriri and Channing, 1975b; Gwatkin and Andersen, 1976; Jagiello *et al.*, 1977; Tsafriri *et al.*, 1977; Hillensjö *et al.*, 1978a). This inhibitory effect is not species specific; thus human FF inhibited the maturation of porcine (Hillensjö *et al.*, 1978a) and rat oocytes (Chari *et al.*, 1983), bovine FF inhibited hamster oocytes (Gwatkin and Andersen, 1976) and porcine FF inhibited the maturation of rat oocytes (Tsafriri *et al.*, 1977). The OMI activity in porcine FF appears to decline in the course of follicular growth. This was demonstrated by assaying FF freshly collected from small, medium and large follicles (Stone *et al.*, 1978) or fluid collected from pigs on specified days of their reproductive cycle (van de Wiel *et al.*, 1983). Similarly, in FF collected from women participating in an *in vitro* fertilization–embryo transfer programme, the OMI activity was significantly lower in follicles yielding mature and fertilizable oocytes as compared with follicles yielding immature or atretic oocytes (Channing *et al.*, 1983; Hillensjö *et al.*, 1985).

OMI purification and characterization

The work on purification and chemical characterization of follicular OMI activity is hampered by difficulties, particularly in two areas; the assay systems used to monitor purification and insufficient resolution of the purification procedures. The *in vitro* assays employed for OMI activity are slow and cumbersome and their relevance to physiological regulation of oocyte maturation is sometimes questionable. Moreover, the lack of standard preparations and assay procedures makes it very difficult to compare results of different research teams. The most commonly employed sources of OMI, follicular fluid and ovarian extracts, are extremely complex and vary as a result of subtle physiological and endocrine conditions and, therefore, impose high demands of the purification procedure. Finally, the active factor isolated from follicular fluid or other biological sources may not be the potent native hormone but a chemically modified product retaining some of the original activity or mimicking the action of an unrelated hormone.

Pig oocytes from either medium or large follicles were frequently employed for bioassay of OMI (Tsafriri and Channing, 1975b; Hillensjö *et al.*, 1978a; Stone *et al.*, 1978). This assay requires a steady source of a large number of porcine ovaries, a long incubation period (42–44 h) and fixation and staining of the ova after the incubation. Rat (Tsafriri *et al.*, 1977) and mouse (Downs and Eppig, 1984, 1985) oocytes were also used for assaying OMI. Murine oocytes are widely available and the culture period is much shorter (up to 4–6 h). Nevertheless, OMI assays involving mammalian ova are cumbersome and tedious, require experienced personnel and can be applied only to a limited number of samples. Inhibitory effects of human follicular fluid on maturation of amphibian oocytes was demonstrated (Schuetz and Rock, 1982; Cameron *et al.*, 1983). It is postulated that the inhibition resulted from the presence of OMI in this fluid and hence it may be employed for purification of OMI.

At the present, two biochemically different entities are proposed as putative follicular OMI, a yet unidentified peptide and hypoxanthine or other purine bases or metabolites. These studies will be reviewed in the following sections.

OMI as a peptide

OMI passed through an Amicon PM-10 filter which has a molecular weight cut-off 10 000. Chromatography of the concentrated filtrate on Sephadex G-25 (Tsafriri *et al.*, 1976d; Stone *et al.*, 1978) revealed that OMI had a molecular weight < 2000 and that two peaks of activity were present. FF lost OMI activity when it was treated with trypsin but retained its activity on freezing and thawing, treatment with charcoal and when it was heated to 60°C for 20 min (Tsafriri *et al.*, 1976d).

Unpublished experiments (S.H. Pomerantz, personal communication) showed that bacterial protease from *Streptomyces griseus* (Sigma) destroyed all of the OMI activity of a partially purified Biogel P2 fraction. Treatment of the same fraction with trypsin left no significant activity. These results are all consistent with OMI being a peptide of low molecular weight. OMI from bovine FF and granulosa cells appears to have similar properties (Gwatkin and Andersen, 1976; Sato *et al.*, 1978; Sato and Koide, 1984).

After lyophilization to dryness of the PM-10 filtrate, OMI could be extracted with acetic acid dissolved in methanol (Channing *et al.*, 1982). This step was then followed by gel filtration chromatography on Sephadex G-25 or Biogel P2 (S.A. Pomerantz and P.A. Bilello, unpublished experiments). After gel filtration, ion-exchange chromatography on OM-Sephadex and DEAE-Sephadex was applied (Pomerantz *et al.*, 1979; Channing *et al.*, 1982). These steps showed OMI to be an acidic compound since it was bound to DEAE – but not OM-Sephadex. However, these procedures resulted in a modest purification and a poor yield, and a lengthy period of time was required.

The current procedure exploits the acidic nature of OMI. First, pig FF is filtered through an Amicon PM-10 filter, and the filtrate passed through a YCO5 filter. The retentate is diluted to 50 mosmol/kg and passed onto a column of QAE-Sephadex. This strongly basic ion-exchange resin binds OMI; the latter is eluted from the column with a steep gradient of ammonium bicarbonate. Two peaks of OMI activity are detected by assays with pig oocytes. The active peaks combined are then treated by chromatography on reversed-phase and gel filtration high performance liquid chromatography (HPLC). Two active fractions were obtained. The fold purification from FF reaches about 20 000 with a combined recovery of about 34% (Pomerantz *et al.*, 1989).

The inhibition of spontaneous maturation of rat, but not mouse, oocytes by Müllerian inhibiting substance (MIS) was reported by Takahashi *et al.* (1986). However, by using a more purified preparation (Picard and Josso, 1984; Picard *et al.*, 1986) we could not detect OMI-like activity on rat oocytes (Tsafriri *et al.*, 1988). It appears, therefore, that the observed OMI activity should be ascribed to a contaminant of the MIS preparation. The inhibition of maturation by human recombinant MIS was dependent upon the presence of a detergent, Nonidet P-40 (Ueno *et al.*, 1988), which by itself may suppress maturation (Tsafriri *et al.*, 1991b). It remains to be determined whether MIS has OMI activity independent of the detergent and, if so, whether it is involved in the regulation of meiosis *in vivo*.

Two additional ovarian hormones were recently implicated in the regulation of oocyte maturation: inhibin (O *et al.*, 1989) and the 35-kDa, but not 32-kDa follistatin (see Esch *et al.*, 1987, for follistatin sequence; Buscaglia *et al.*, 1989). Furthermore, an antiserum towards the 27 amino acid carboxy terminus of the 35-kDa follistatin neutralized the OMI activity of porcine FF (Buscaglia *et al.*, 1989) or of partially purified OMI standard preparations (our unpublished observations). Thus, it is possible that the OMI peptide is a fragment of the carboxy terminus of 35-kDa follistatin. The very acidic nature of partially purified OMI (Pomerantz *et al.*, 1989) from porcine FF and of the carboxy terminus of 35-kDa follistatin support this possibility. Further studies are required in order to confirm these interesting observations and to identify OMI as any of the known ovarian hormones or their fragments.

Inhibition of oocyte maturation by purine bases and their metabolites

By the use of mouse oocytes as an assay system, an inhibitory effect of porcine FF was demonstrated and this inhibition was potentiated by cyclic AMP (Downs and Eppig, 1984, 1985). Downs *et al.* (1985) reached the

conclusion that hypoxanthine is the predominant low molecular weight component of porcine FF that inhibits mouse oocyte maturation. This conclusion is based on the findings that the potent inhibitory fraction (i) had an absorption maximum (250 nm) identical to that of hypoxanthine; (ii) had a retention time on HPLC of pure hypoxanthine; (iii) exerted inhibition of oocyte maturation identical to that exhibited by a commercial preparation of hypoxanthine; and (iv) hypoxanthine concentration of about 1.41 mM could account for the majority of the inhibitory activity of porcine FF1 on mouse oocytes. The concentrations of hypoxanthine (2–4 nM) and of adenosine (0.35–0.70 mM) in mouse FF could account for inhibition of oocyte maturation, since even lower concentrations of these purines when combined together inhibited the spontaneous maturation of mouse oocytes during a 24 h incubation period *in vitro* (Eppig *et al.*, 1985). Nevertheless, the same authors were unable to detect a reduction in FF hypoxanthine and adenosine concentration 2 h after hCG administration, i.e. just before GVB. It is possible that the concentration of these purines in FF does not necessarily reflect their levels within the oocyte, which are probably more relevant for maintenance of meiotic arrest. Hypoxanthine and adenosine were not detected in bovine FF, in spite of the presence of a low molecular weight component which was inhibitory for ovum maturation (J.J. Eppig, personal communication). Guanosine and hypoxanthine inhibited, dose-dependently, the spontaneous maturation of rat oocytes, with guanosine the more effective, 50 μM inhibiting resumption of meiosis to the same extent as 2 mM hypoxanthine, and FSH potentiated the inhibitory action of both purines (Billig *et al.*, 1985). By contrast, in another study, 30 μM adenosine, but not adenine, inosine, hypoxanthine or guanosine, synergized with FSH in inhibiting maturation of rat oocytes (J.G.O. Miller and Behrman, 1986). Purine bases were demonstrated to modulate oocyte levels of cAMP, most probably by inhibiting cAMP phosphodiesterase activity (Downs *et al.*, 1989). Furthermore, microinjection of oocytes with an inhibitor of cAMP-dependent protein kinase (PKO) induced the resumption of meiosis in oocytes cultured in medium containing hypoxanthine or guanosine (Eppig, 1989). It seems, therefore, that purines affect resumption of meiosis by raising oocyte cAMP to levels inhibitory for meiosis (see review by Törnell *et al.*, 1991). Such action of purine bases does not exclude the possibility of other follicular factors suppressing in parallel the resumption of meiosis. Indeed, Downs *et al.* (1985) observed an additional inhibitory fraction in FF which was not removed by charcoal extraction. The resistance of OMI activity to proteolysis cannot be taken as conclusive evidence that OMI is not a peptide. A small peptide may be a very poor substrate for proteases and/or the appropriate sensitive peptide bonds may not be present. Hence, the final identification of OMI as a purine base or a peptide must await confirmation in several mammalian test systems. Of course, the coexistence of follicular mechanisms suppressing meiosis by a peptide and by purine base(s) is a possibility which cannot be dismissed at the present. We shall further discuss this issue in the next section.

OMI as a physiological regulator of ovum maturation

In order to serve as a physiological regulator of meiosis, a substance has to fulfil the following criteria: (i) it should be present in a compartment relevant to regulation of meiosis at the proper timing; (ii) its action should be reversible with no adverse effects on further stages of ovum maturation, fertilization and embryonic development; (iii) the physiological trigger of resumption of meiosis, gonadotrophin, should be able to either supersede its inhibitory action or cause its

degradation or removal from the relevant compartment. The inhibitory action of porcine FF and of its partially purified fractions could be reversed by either transferring the oocytes after an initial 24-h incubation with the inhibitor to a medium without OMI (Stone *et al.*, 1978; Hillensjö *et al.*, 1979a,b) or by the addition of an antibody prepared against the low molecular weight fraction of porcine FF (Tsafriri, 1979). Likewise, the inhibition of maturation of mouse oocytes by hypoxanthine and adenosine was completely reversible by withdrawal of the purines (Eppig *et al.*, 1985). Oocytes arrested *in vitro* and matured after withdrawal of the drug could be fertilized and developed to the expanded blastocyst stage. Addition of FSH further increased development to two-cell and blastocyst stages and 11% of transferred morulae produced live young as compared with 14% in control oocytes which were not arrested by purines (Downs *et al.*, 1986a). Thus, the inhibition of meiosis, by both the apparently peptide OMI from porcine FF and of hypoxanthine and adenosine appears, not to be due to a toxin and irreversible action.

In vivo or in follicle-enclosed oocytes *in vitro*, resumption of meiosis is induced by LH. Hence the ability of LH (or other gonadotrophins) to overcome the inhibition of meiosis by FF or by co-culture with granulosa cells lends support for the physiological role of OMI in the regulation of meiosis. It was found that the addition of LH to cultures of porcine oocytes with porcine FF or its purified fractions overcame their inhibition of meiosis (Tsafriri and Channing, 1975b). Also, the inhibitory action of bovine or hamster FF (Gwatkin and Andersen, 1976), of porcine FF and of rat granulosa cell conditioned medium as well as co-culture with rat granulosa cells (Tsafriri *et al.*, 1977; Tsafriri, 1979) were all alleviated by the addition of LH, closely resembling the regulation *in vivo* of oocyte maturation. Likewise, recent studies demonstrated the ability of FSH to induce resumption of meiosis in the continued presence of hypoxanthine (Eppig and Downs, 1986). Furthermore, perturbants of purine metabolism induced resumption of meiosis *in vitro* in meiotically arrested oocytes in the presence of hypoxanthine (Downs *et al.*, 1986b) or *in vivo* in PMSG-primed immature mice (Downs and Eppig, 1986). Thus, mycophenilic acid and bredinin, drugs inhibiting the enzyme inositol monophosphate (IMP) dehydrogenase and hence the conversion of hypoxanthine product IMP to guanyl and/or xanthyl compounds, induced resumption *in vivo* and *in vitro*. Also, azastene, an inhibitor of *de novo* purine synthesis, induced meiosis in a dose-dependent manner. These data support the essential role of guanyl and/or xanthyl derivatives in the maintenance of meiotic arrest within the follicle.

Follicular OMI activity has been demonstrated by several laboratories and by a variety of approaches. Some investigators, however, were unable to confirm the presence of OMI activity in various follicular preparations. These varying findings may be related, at least in part, to the low OMI activity of follicular fluid and of granulosa cell cultures; the finding that fluid and granulosa cells from large follicles are devoid of inhibitory activity; the relative instability of OMI and to differences in the methods of oocyte collection and culture. Furthermore, some of the groups that initially have reported negative results confirmed the inhibition of meiosis in a later study. Thus, bovine FF was found to inhibit the maturation of bovine oocytes (Sirad and First, 1988) in contrast to an earlier report (Leibfried and First, 1980a,b). Likewise, both FF and granulosa cells were found to inhibit the maturation of hamster oocytes, the granulosa cells persistently and FF only transitorily (Racowsky and Baldwin, 1989), contrary to earlier reports (see Racowsky and McGaughey, 1982a).

Collectively, the studies reviewed above clearly support the role of local paracrine mechanisms in regulation of oocyte maturation. Both the yet unidentified peptide OMI

and purine bases fulfil several criteria of physiological regulators of meiosis. In the following sections we shall review additional mechanisms involved in oocyte maturation. Such multiple mechanisms afford the notion that more than one type of molecule is involved in the maintenance of meiotic arrest.

(e) Other factors involved in the regulation of meiosis

Steroids

Steroids as follicular inhibitors of meiosis Studies testing the effect of steroids on spontaneous maturation of isolated oocytes led to conflicting results regarding their putative role in arrest of meiosis (reviewed by McGaughey, 1983; Eppig and Downs, 1984; Tsafriri, 1985; Schultz, 1991). For example, authors from one laboratory reported significant inhibitory action of testosterone (Rice and McGaughey, 1981), while in a later study no such inhibition was observed (Racowsky, 1983). Also, the reported inhibition of meiosis by oestrogen (Richter and McGaughey, 1979) was shown to be exerted only in the absence of added proteins in the medium (Racowsky and McGaughey, 1982b). Since follicular fluid has a high protein content, it has been concluded that oestradiol does not play a direct physiological role in maintaining meiotic arrest (Racowsky and McGaughey, 1982b). The inconsistent effects of steroids on resumption of meiosis render their suggested role in maintaining meiotic arrest doubtful (see also Tsafriri, 1985). Nevertheless, a modulatory action of steroids on resumption of meiosis seems more acceptable. Several steroids, such as oestradiol-17β, testosterone, dihydrotestosterone, or progesterone potentiate the inhibitory action of dibutyryl-cAMP, 8-bromo-cAMP or forskolin (Rice and McGaughey, 1981; Eppig *et al.*, 1983; Racowsky, 1985a). An antioestrogen, tamoxifen, counteracts the synergistic inhibition of GVB elicited by forskolin and oestradiol (Racowsky, 1985b). The modulatory actions of steroids on meiosis are exerted probably directly on the oocyte (Eppig and Downs, 1984) and may be related to their ability to inhibit oocyte cyclic nucleotide phosphodiesterase activity (Kaji *et al.*, 1987; see also Schultz, 1991).

Do steroids mediate the meiosis-inducing action of LH? Following a gonadotrophic stimulus, a prompt rise in follicular steroidogenesis, especially progesterone, occurs (see reviews by Lindner *et al.*, 1974; Channing and Tsafriri, 1977). The role of progesterone in mediation of the meiosis-inducing action of gonadotrophin is well established in fish and amphibia (Schuetz, 1985). Therefore, the rise in follicular steroidogenesis could admit the involvement of progesterone in triggering resumption of meiosis in mammals.

Addition of steroids to the culture medium did not induce the maturation of bovine, porcine (Foote and Thibault, 1969) or rat (Tsafriri *et al.*, 1972a) follicle-enclosed oocytes. Furthermore, suppression of follicular steroidogenesis by specific inhibitors, which reduced progesterone to a level below that seen in LH-free control follicles in which germinal vesicle breakdown fails to occur, did not impair the ability of LH (or PGE_2) to induce GVB in rat follicle-enclosed oocytes (Lieberman *et al.*, 1976). Similar results were obtained by Moor (1978) in the sheep. Dissociation of the steroidogenic and meiosis-inducing action of LH was observed under two additional conditions: (i) during selective inhibition of RNA synthesis by actinomycin D (Tsafriri *et al.*, 1973; Lieberman *et al.*, 1975) and (ii) during inhibition of aerobic glycolysis by iodoacetate (Tsafriri *et al.*, 1976b). In both instances, progesterone production was inhibited, whereas germinal vesicle breakdown went on undisturbed. Thus, the abolition or suppression of LH-induced changes in follicular steroidogenesis by several inhibitors fails to prevent the action of LH on GVB, while none of the

Table 1.3 Oocyte cAMP levels and their relationship to resumption of meiosis (data from Dekel, 1987)

Treatment	*Meiotic stage*	*cAMP (fmol per denuded oocytes)*
(a) Denuded oocytes		
Freshly isolated from the ovary	Immature	1.23±0.3
Freshly isolated from the oviduct	Mature	0.81±0.2[a]
Incubated for 2 h	Mature	0.80±0.1[a]
Incubated for 2 h with IBMX (0.2M)	Immature	1.21±0.1
(b) Cumulus–oocyte transfer of cAMP[b]		
Cultured denuded	Immature	1.19±0.47
Cultured within intact cumulus oocyte complex	Immature	3.51±0.53[c]

[a] $P < 0.01$ *vs.* immature oocytes.
[b] Denuded or cumulus-enclosed oocytes were incubated for 1 h with 100 μM forskolin + 0.2 mM IBMX.
[c] $P < 0.01$ *vs.* oocytes cultured denuded.

steroids examined was able to substitute for LH in initiating meiosis. It appears, therefore, that steroids do not mediate the action of LH on resumption of meiosis in mammals. Nevertheless, this does not preclude an essential role of steroids in the acquisition of the ability of oocytes to undergo meiosis (see above) or to complete the process and to develop normally after fertilization (Moor *et al.*, 1980b; see Osborn and Moor (1983b) and Osborn *et al.* (1986); see also Chapter 2, this volume).

Cyclic AMP

Many of the agents inducing the maturation of follicle-enclosed oocytes *in vitro* also stimulate production of cAMP (Tsafriri *et al.*, 1972a; Lindner *et al.*, 1974). Injection of the cAMP derivative dibutyryl cAMP (dbcAMP) into the follicular antrum (Tsafriri *et al.*, 1972a) or short-term exposure of follicles to 8-bromo-cAMP (Hillensjö *et al.*, 1978b), dbcAMP or isobutyl methyl xanthine (IBMX) (Dekel *et al.*, 1981) triggered the resumption of meiosis. By contrast, the continuous presence of cAMP derivatives or several inhibitors of phosphodiesterase prevented the induction of meiosis by LH (Hillensjö *et al.*, 1978b; Dekel *et al.*, 1981). The spontaneous maturation of isolated oocytes from mice and rats was prevented in the presence of cAMP derivatives or phosphodiesterase inhibitors (Cho *et al.*, 1974; Hillensjö, 1976; Dekel and Beers, 1978; Hillensjö *et al.*, 1978b).

It seems, therefore, that enhanced production of cAMP in the somatic cell compartment of the follicle is involved in the mediation of the meiosis-inducing action of LH, while elevated cAMP in the oocyte inhibits the resumption of meiosis. It has been suggested that cAMP serves as a physiological regulator of meiosis in mammalian oocytes (Lindner *et al.*, 1974; Schultz and Wassarman, 1977b; Dekel and Beers, 1978, 1980; Moor *et al.*, 1980a; Dekel *et al.*, 1981; Dekel and Sherizly, 1983; Sherizly *et al.*, 1988). Indeed, a decrease in murine oocyte cAMP levels precedes resumption of meiosis, and when this decrease is prevented resumption of meiosis is blocked or substantially delayed (Table 1.3; Schultz *et al.*, 1983a,b; Vivarelli *et al.*, 1983; Aberdam *et al.*, 1987).

Likewise, incubation of rat oocytes with an invasive adenylate cyclase from bacteria of the genus *Bordetella*, elevated oocyte cAMP levels and inhibited resumption of meiosis, while removal of the enzyme resulted in a drop of oocyte cAMP levels and resumption of meiosis (Dekel *et al.*, 1985; Aberdam *et al.*,

1987). These results support the notion that elevated levels of oocyte cAMP maintain meiotic arrest and that an intraoocyte drop in cAMP allows the resumption of meiosis. In ovine oocytes an increase, rather than decrease, in cyclic AMP levels was observed at 1 and 12–18 h after gonadotrophic stimulation of explanted follicles *in vitro* (Moor and Heslop, 1981). Nevertheless, the relatively long interval between the meiosis-inducing stimulus and GVB in ovine oocytes still allows for a transient decrease in oocyte cAMP levels which has not been detected.

Studies employing forskolin, a potent and reversible activator of adenylate cyclase, provide further support for this hypothesis. Addition of forskolin to the medium induced resumption of meiosis in rat follicle-enclosed oocytes (Dekel and Sherizly, 1983). Likewise, perfusion of rabbit ovaries with forskolin resulted in the resumption of meiosis (Yoshimura *et al.*, 1992a,b). By contrast, forskolin inhibited the spontaneous maturation of cumulus-enclosed oocytes and had no effect on the maturation of denuded oocytes (Dekel *et al.*, 1984a; Racowsky, 1984; Yoshimura *et al.*, 1992a,b). Similar results were obtained when cholera toxin was used (Dekel and Beers, 1980). These results seem to indicate that (i) activation of adenylate cyclase in the somatic compartment of the follicle induces resumption of meiosis; (ii) activation of adenylate cyclase in the cumulus–oocyte complex results in inhibition of meiosis; (iii) the rat oocyte is devoid of adenylate cyclase or the level of cAMP produced by the oocyte is not sufficient to maintain meiotic arrest. Indeed, evidence for transfer of cyclic AMP from the cumulus cells to the ovum has been obtained in rat, mouse, hamster and porcine oocytes (Table 1.3; Dekel *et al.*, 1984a; Racowsky, 1984, 1985a–c; Bornslaeger and Schultz, 1985; Sherizly *et al.*, 1988). Studies with follicle-enclosed hamster and mouse oocytes support the same conclusions in spite of somewhat divergent results. In these species, continuous exposure to forskolin did not induce resumption of meiosis and, furthermore, it blocked LH-induced maturation. By contrast, transient exposure to forskolin triggered maturation of follicle-enclosed oocytes in the hamster (Hubbard, 1985) and the mouse (N. Hashimoto *et al.*, 1985). The inhibition of maturation of hamster and mouse follicle-enclosed oocytes by forskolin is probably associated with the sustained increase in oocyte cAMP observed in these species, as compared with the rat (Ekholm *et al.*, 1984; Racowsky, 1984). These studies leave little doubt regarding the important role of oocyte cAMP in the resumption of meiosis in mammals. However, the question of whether the oocyte synthesizes a sufficient amount of cAMP to inhibit meiosis, and whether cAMP is transferred from the cumulus to the oocyte, was answered by contrasting experimental results. Thus, while forskolin did not affect the resumption of meiosis in denuded oocytes of the rat (Dekel *et al.*, 1984a; Racowsky, 1984), and only partially in the hamster (Racowsky, 1985b), in other studies it was inhibitory in mouse (Schultz *et al.*, 1983a; Urner *et al.*, 1983), rat (Olsiewski and Beers, 1983) and pig (Racowsky, 1985a) denuded oocytes. The varying results may be related, at least in part, to species and strain differences, as well as to the experimental procedures explored. The interpretation of the results may be complicated by the effects on oocyte adenylate cyclase and phosphodiesterase activity (see Bornslaeger and Schultz, 1985).

The inhibition of oocyte maturation by IBMX supports the notion that phosphodiesterase plays a role in regulation of meiosis. Furthermore, the degree of suppression of phosphodiesterase activity by three pharmacological inhibitors of the enzyme in oocyte extracts correlated with their ability to inhibit GVB (Bornslaeger *et al.*, 1984). The activity of oocyte phosphodiesterase is most likely modulated by calmodulin, which constitutes 0.3% of total oocyte protein (Bornslaeger *et al.*, 1984). That a calmodulin-dependent step

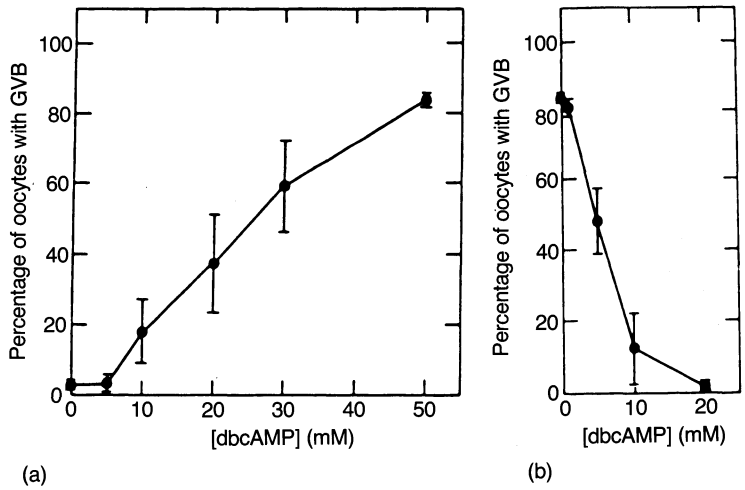

Figure 1.8 Effect of dbcAMP on maturation of follicle-enclosed rat oocytes. (a) Induction of oocyte maturation: preovulatory follicles were explanted on the day of pro-oestrus and incubated in the presence of the indicated concentrations of dbcAMP. After 20 h the follicles were rinsed (×4) and incubated further for 5 h in a medium without dbcAMP, the oocytes isolated and the presence of GV observed by interference contrast microscopy. The mean ±SEM of three different experiments is presented; at least 100 oocytes were examined at each dbcAMP concentration. (b) Inhibition of oocyte maturation: preovulatory follicles were incubated in the presence of 5 μg/ml ovine LH (NIH) with or without the indicated concentration of dbcAMP. After 20 h the oocytes were isolated and GVB scored. Each point is based on four different experiments. Other details as in (a). (From Dekel, 1987.)

is involved in maturation is suggested by the inhibition of maturation by calmodulin inhibitors, trifluoperazine, calmidizolium, W7 and W13. Nevertheless, since W7 at concentrations that inhibit maturation does not prevent the decrease in oocyte cAMP it seems that a calmodulin-mediated process, other than phosphodiesterase, is involved in oocyte maturation. This could be a calmodulin-modulated protein phosphatase (see Schultz, 1985). The involvement of phosphorylation in control of meiosis will be discussed in the next section. Collectively, the essential role of oocyte cAMP in the regulation of mammalian meiosis seems well established. The observed synergism of cAMP and OMI or purines in inhibiting spontaneous maturation *in vitro* of isolated oocytes (Eppig and Downs, 1984; Törnell *et al.*, 1984; Tsafriri and Pomerantz, 1984) is suggestive of interaction of cAMP and follicular OMI in the control of oocyte maturation. This concept further emphasizes the multifactorial control of resumption of meiosis.

The dual role of cAMP in the regulation of oocyte maturation was elegantly demonstrated by Dekel *et al.* (1988). Inhibition of resumption of meiosis in follicle-enclosed ova stimulated by LH or in cumulus-enclosed ova maturing spontaneously was achieved by continuous culture in the presence of IBMX (ED_{50}= 0.03 mM) or dbcAMP (ED_{50}= 5.0 mM). By contrast, induction of ovum maturation in follicle-enclosed oocytes was achieved by transient exposure to IBMX (ED_{50}= 0.13 mM) or dbcAMP (ED_{50}= 25 mM; Figure 1.8).

Thus, the induction of maturation is achieved by much higher concentrations of the phosphodiesterase inhibitor or the cyclic nucleotide analogue. These findings show that basal levels of follicular cAMP maintain meiotic arrest, while LH-stimulated surge of the nucleotide reaching higher levels is

required for the maturation-inducing action of the hormone. Furthermore, these divergent actions of the nucleotide are related to two different target cells; the inhibitory action is exerted at the oocyte, while the surge of cAMP in the somatic granulosa and cumulus cells leads to the resumption of meiosis.

Studies in the hamster implicate also cyclic GMP (or the cAMP/cGMP ratio) in the regulation of oocyte maturation. Thus, 8-bromocyclic GMP inhibited the spontaneous maturation of hamster cumulus-enclosed oocytes and this was overcome by LH (Hubbard and Terranova, 1982). Hamster follicular levels of cyclic GMP were decreased after gonadotrophic stimulation, and LH-induced maturation in hamster follicle-enclosed oocytes was enhanced by dbcGMP (Hubbard and Terranova, 1982).

Role of cumulus cells and of cumulus–oocyte communication

The cumulus cells appear to have an important role in the regulation of meiosis. Whereas OMI from porcine follicular fluid inhibited the resumption of meiosis by oocytes cultured within their intact cumuli, it did not interfere with the maturation of denuded oocytes of the pig (Hillensjö *et al.*, 1979b) or rat (Tsafriri and Bar-Ami, 1982). By contrast, porcine FF attenuated maturation of mouse denuded oocytes. Nevertheless, this study also showed that the suppression of resumption of meiosis in mouse denuded oocytes was consistently less than in cumulus-enclosed oocytes (Downs and Eppig, 1984). The uptake and metabolism of hypoxanthine and adenosine were augmented in intact cumulus complexes as compared with denuded oocytes (Downs *et al.*, 1986b). It therefore appears that the inhibitory action of OMI upon meiosis is exerted, at least partially, through the mediation of cumulus cells.

The cumulus cells are, apparently, involved also in the mediation of the meiosis-inducing action of LH. Whereas it was not possible to demonstrate specific receptors for LH on the oocyte, specific LH/hCG receptors were demonstrated on cumulus cells (Lawrence *et al.*, 1980; Channing *et al.*, 1981). This finding was further supported by the response of the whole oocyte–cumulus complex to gonadotrophins, involving enhanced steroidogenesis (Nicosia and Mikhail, 1975; Hillensjö *et al.*, 1980), lactate production (Billig *et al.*, 1983), cumulus mucification (Dekel and Kraicer, 1978; Hillensjö and Channing, 1980) and activation of adenylate cyclase (Eppig *et al.*, 1983). LH accelerated resumption of meiosis in mouse and rat oocytes pretreated with dbcAMP and IBMX, and LH was effective only in oocytes cultured within their cumulus complex but not in denuded oocytes (Dekel and Beers, 1978, 1980; Freter and Schultz, 1984). It was suggested that acceleration of meiosis by LH is mediated by an increase in cumulus cell cAMP levels, which promotes a decrease in maturation inhibitors. The finding that the prevention of the LH-induced increase in cAMP with *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, also inhibited the LH-induced acceleration of oocyte maturation lends support to this notion (Freter and Schultz, 1984). Germinal vesicle breakdown in rat (Tsafriri, 1979), mouse (Eppig, 1982; Salustri and Siracusa, 1983), and rabbit (Thibault *et al.*, 1976) oocytes maturing spontaneously occurs earlier than GVB in follicle-enclosed oocytes stimulated by gonadotrophin. This suggests that, by isolating an oocyte from its follicle, some regulatory step(s) essential for triggering meiosis *in vivo* is bypassed. This may include merely the removal of the inhibitory signal (OMI) by decreased synthesis, increased degradation or a combination of both or, alternatively, the generation of a positive signal which triggers the resumption of meiosis. Some of these alternative regulatory mechanisms of meiosis were reviewed and compared in mammalian, amphibian and echinoderm oocytes (Schuetz, 1985). In

immature oocytes the cumulus cells are closely apposed to the zona pellucida, which is studded with numerous cytoplasmic projections, whereas the cumulus cells encircling mature oocytes are loosely organized and very few projections are present in the zona pellucida (Zamboni, 1972; Szöllösi, 1975; Szöllösi *et al.*, 1978). Also, ionic coupling and transfer of molecules were maximal before gonadotrophic stimulation and decreased gradually afterwards (Gilula *et al.*, 1978; Sherizly *et al.*, 1988). These findings led to the hypothesis that the release of the oocyte from follicular suppression of meiosis is a result of the uncoupling of cumulus–oocyte communication, possibly induced by the ovulatory hormone (Lindner *et al.*, 1974; Dekel and Beers, 1978, 1980; Gilula *et al.*, 1978; Moor *et al.*, 1980a). The influence of LH on the resumption of meiosis may be secondary to its action on the sequestration of the oocyte. However, several of the initial observations were in contradiction with this suggestion. Thus, the disruption of oocyte–cumulus cell communication followed, rather than preceded, germinal vesicle breakdown. This was inferred from the observed reduction of the transport of choline or uridine to ovine, mouse, porcine and rat oocytes (Moor *et al.*, 1980a; Eppig, 1982; Racowsky, 1984; 1985a) and from the termination of ionic coupling of the cumulus cells and the oocyte in the rat (Dekel *et al.*, 1981).

Similarly, there was no consistent relation between cumulus dissociation and resumption of meiosis in porcine oocytes (Gérard *et al.*, 1979). Furthermore, it was possible to dissociate between germinal vesicle breakdown and the disruption of cumulus–oocyte communication as revealed by choline transport; whereas a low dose of FSH (50 ng/ml) suppressed intercellular coupling but did not induce resumption of meiosis, LH (100–500 ng/ml) induced resumption of meiosis but not disruption of coupling (Moor *et al.*, 1981).

Recent studies demonstrated that transfer of amino acids (Colonna and Mangia, 1983) and uridine (Salustri and Siracusa, 1983) to the oocyte is linearly dependent on their uptake by cumulus cells. Hence, under conditions entailing changes in precursor uptake by the cumulus cells, expressing oocyte uptake as a fraction of that of the cumulus appears to be a more proper index of oocyte–cumulus metabolic coupling than the comparison of absolute values of label incorporation used in the above-mentioned studies. Indeed, when the relative uptake was examined, there was a strict correlation between the resumption of meiosis and oocyte–cumulus uncoupling in mouse oocytes (Salustri and Siracusa, 1983). Furthermore, when rat follicle-enclosed oocytes were examined at various time intervals after LH/hCG stimulation, a 50% reduction in uridine uptake was observed already 1 h after the hormonal stimulus *in vivo* or *in vitro*, when GVB is still present in all oocytes (Dekel *et al.*, 1984b; Dekel, 1986; Sherizly, *et al.*, 1988). Similarly, a reduction in oocyte–cumulus dye and metabolic coupling was observed in hamster oocytes concomitantly with GVB (Racowsky and Satterlie, 1985). Also, when resumption of meiosis was triggered by epidermal growth factor (EGF), oocyte–cumulus communication was reduced prior to GVB in rat follicle-enclosed oocytes (Dekel and Sherizly, 1985). In a more recent study, it was demonstrated that heptanol, a seven-carbon alcohol that blocks cell-to-cell communication in various experimental systems, including the cumulus–oocyte complex, reduced intraoocyte concentrations of cAMP and promoted maturation of rat follicle-enclosed oocytes (Dekel and Piontkewitz, 1991).

Quantitative analysis of freeze-fractured rat cumulus–oocyte complexes revealed a dramatic decrease in the net area of cumulus gap junctions following the ovulatory stimulus and prior to GVB (W.J. Larsen *et al.*, 1986), thus providing structural basis for the observed reduction in cumulus–oocyte com-

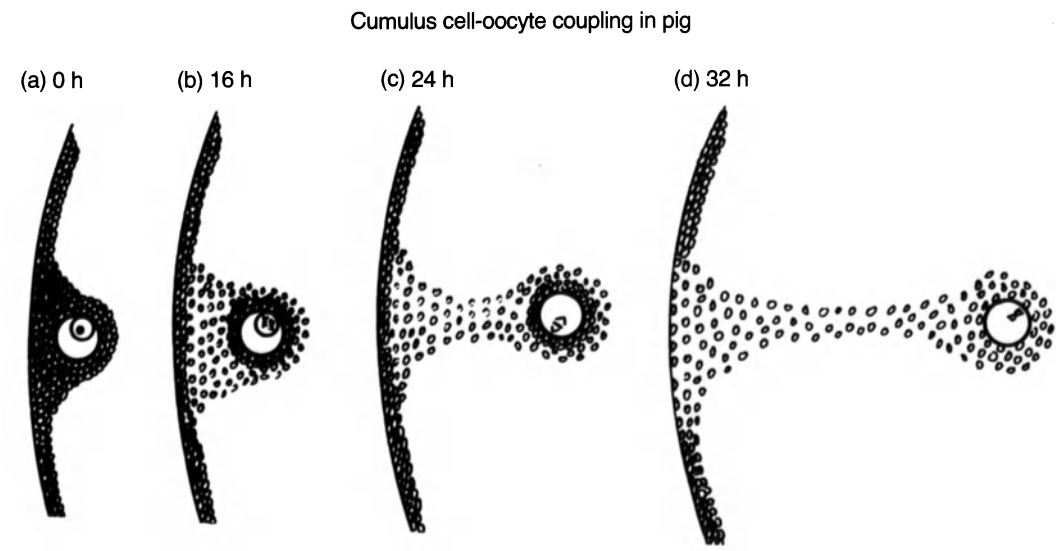

Figure 1.9 Preovulatory changes in the granulosa cell–oocyte complex in pigs. The first signs of cumulus expansion and matrix deposition are observed 16 h after hCG administration in the area of the connection between the cumulus and mural granulosa cells (b). (From Motlik *et al.*, 1986.)

munication. An additional consideration regarding oocyte–follicle cell communication was raised in a recent study by Motlik *et al.* (1986). Examination of the changes in association between cumulus oophorus and mural granulosa in follicles from PMSG, hCG-treated pigs, revealed deposition of mucous matrix already 16 h after hCG stimulation, prior to GVB, which occurs 20–24 h after hCG (Figure 1.9; Motlik and Fulka, 1976).

The importance of mural granulosa–cumulus coupling in regulation of meiosis has been suggested already by Foote and Thibault (1969). Racowsky *et al.* (1989) observed a significant progressive decrease in gap junction area with increasing percentage of oocytes committed to mature after stimulation of ovulation by hCG in the membrana granulosa underlying the cumulus cell stalk in the hamster.

Collectively, these data are compatible with the suggested communication of an inhibitory signal from the somatic cell compartment of the follicle to the oocyte. The rate of transfer of the inhibitory signal may be modulated at several points: oocyte–cumulus, cumulus–cumulus or cumulus–mural granulosa or even granulosa–granulosa junctions. At the present, there is sufficient evidence supporting the transfer of cAMP to the oocyte. Further studies are required to determine whether other regulators of meiosis are transmitted to the ovum or their effect is exerted at the level of the follicular somatic compartment.

Protein phosphorylation

The detailed mechanism(s) by which oocyte cyclic AMP maintains meiotic arrest is not yet known. Nevertheless, since cAMP acts in many eukaryotic systems by activating cAMP-dependent protein kinase (PK-A), a model involving phosphorylation–dephosphorylation of an oocyte protein(s) was suggested (Bornslaeger *et al.*, 1986, see also Schultz, 1991; Tsafriri and Dekel, 1993). This model (Figure 1.10) proposes that a phosphoprotein X-P, a substrate of PK-A, maintains meiotic arrest.

A decrease in PK-A activity caused by a decrease in oocyte cAMP concentration

would, due to phosphoprotein phosphatase activity, result in a net dephosphorylation of X-P; the dephosphorylated protein, X, would then promote resumption of meiosis. In support of this model, an increase in protein phosphorylation–dephosphorylation during maturation of mouse (Schultz *et al.*, 1983a; Bornslaeger *et al.*, 1986; Endo *et al.*, 1986) and sheep oocytes (Crosby *et al.*, 1984; Moor and Crosby, 1986) was observed. Furthermore, microinjection into oocytes of the heat-stable inhibitor of P-A, PKI, induced GVB in the presence of dbcAMP or IBMX. Conversely, microinjection of the catalytic subunit of PK-A maintained meiotic arrest in a dose-dependent manner, provided that PKI was not co-administered (Bornslaeger *et al.*, 1986). Addition of cyclic adenosine 3,5-pyrophosphate, an inhibitor of protein kinase, blocked the spontaneous maturation of mouse oocytes *in vitro*. Furthermore, a fraction of bovine follicular fluid exhibited both actions, inhibition of protein kinase and resumption of meiosis (Sato *et al.*, 1985). Likewise, a puromycin analogue, 6-dimethylaminopurine, which inhibited protein phosphorylation in mouse oocytes, also blocked the resumption of meiosis, without markedly altering protein synthesis in the oocyte (Rime *et al.*, 1989).

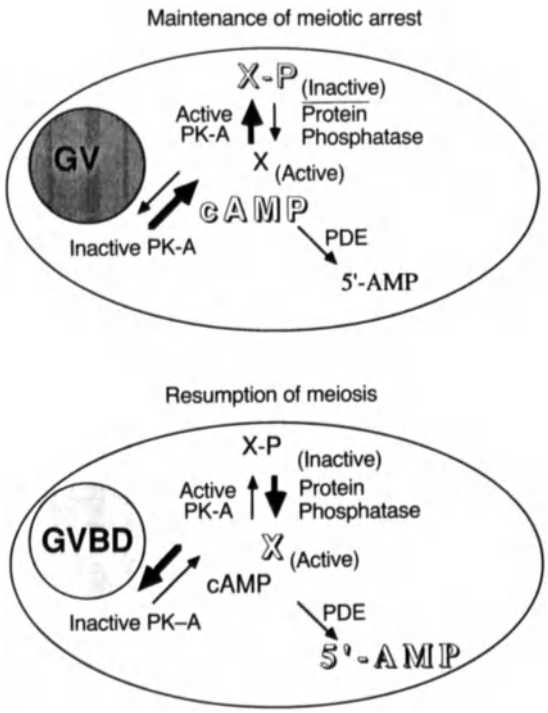

Figure 1.10 Model for maintenance and resumption of meiosis. The bold, open symbols depict the predominating form of a hypothetical maturation-regulating protein 'X' or its phosphorylated inactive form 'XP', as well as cAMP or 5-AMP. (From Bornslaeger *et al.*, 1986.)

Similarly, cAMP-independent kinases may also be involved in regulation of resumption of meiosis. Phorbol esters which activate protein kinase C (PKC) inhibited the spontaneous maturation of mouse oocytes (Urner and Schorderet-Slatkine, 1984; Bornslaeger *et al.*, 1986) and partially inhibited the specific changes in oocyte phosphoprotein metabolism associated with resumption of meiosis (Bornslaeger *et al.*, 1986). Since the phorbol ester did not inhibit the decrease in oocyte cAMP and administration of PKI did not result in GVB in the presence of the phorbol ester, it was concluded that activators of PKC inhibit meiosis by acting distal to a decrease in cAMP-dependent protein kinase, but prior to changes in oocyte phosphoprotein metabolism required for GVB. In contrast, studies with rat oocytes demonstrated the ability of PKC activators to induce meiosis in follicle-enclosed oocytes (Aberdam and Dekel, 1985). Furthermore, the induction of oocyte maturation by GnRH seems to be the result of PKC activation. These findings suggest that PKC may be involved in the control of oocyte maturation. However, the precise physiological role of PKC in the regulation of meiosis remains to be elucidated.

The resumption of meiosis in oocytes of amphibia is triggered by a steroid interacting with a plasma membrane receptor (Sadler and Maller, 1982). This results in a decrease in adenylate cyclase activity (Finidori-Lepicard *et al.*, 1981; Jordana *et al.*, 1981; Sadler and Maller, 1981) and hence decrease in oocyte cAMP levels. The decrease in

oocyte cAMP is followed, among other changes, by increase in protein phosphorylation (see Baulieu and Schorderet-Slatkine, 1983; Maller, 1985). The recent elucidation of MPF and the identification of its catalytic subunit with the product of the *cdc2* (cell division cycle) gene in fission yeast (Gautier *et al.*, 1988, 1989) and the regulatory subunit with cyclins (Murray *et al.*, 1989; Murray and Kirschner, 1989), its conservation throughout eukaryotes and its role in regulation of both mitotic cycle and meiosis may lead to the identification of the mechanisms operating in mammalian ooplasm. In spite of the different stimulus for resumption of meiosis in mammals, and the crucial role of follicular granulosa and cumulus cells in regulation of the meiotic process, it seems most likely that similar, if not identical, mechanisms operate in the ooplasm of amphibian and mammalian oocytes. The demonstration of MPF activity in the cytoplasm of mouse oocytes (H. Hashimoto and Kishimoto, 1988) supports this notion.

Macromolecular synthesis

In rat follicle-enclosed oocytes addition of inhibitors of protein synthesis, but not of RNA synthesis, blocked LH-induced resumption of meiosis (Tsafriri *et al.*, 1973; Lieberman *et al.*, 1975). This effect was most probably exerted in the somatic compartment of the follicle.

In full-grown mouse oocytes low, but significant, incorporation of precursors into nuclear and cytoplasmic RNA *in vivo* (Rodman and Bachvarova, 1976) and *in vitro* (Bloom and Mukherjee, 1972; P.M. Wassarman and Letourneau, 1976) was detected. Nevertheless, this activity is lower in full-grown oocytes than in growing ones. Likewise, a fall in RNA polymerase activity was observed in full-grown oocytes (Moore and Lintern-Moore, 1978). With resumption of meiosis RNA synthesis is reduced to undetectable levels at GVB (Rodman and Bachvarova, 1976; P.M. Wassarman and Letourneau, 1976; Motlik *et al.*, 1984b; Motlik and Fulka, 1986). During oocyte maturation a 50% decline in polyadenylated RNA (Brower *et al.*, 1981; Bachvarova *et al.*, 1985) and a 20% drop in total RNA (Bachvarova *et al.*, 1985) was estimated. In contrast, protein synthesis continues throughout maturation and exhibits marked quantitative and qualitative changes.

The absolute rate of protein synthesis drops by about 25% from 43 to 31 pg/h in maturing oocytes (Schultz *et al.*, 1978). Changes in the pattern of oocyte proteins were detected after GVB by two-dimensional gel electrophoresis in several mammalian species (sheep, Warnes *et al.*, 1977; pig, McGaughey and Van Blerkom, 1977; rabbit, Van Blerkom and McGaughey, 1978; and mouse, Schultz and Wassarman, 1977a; Van Blerkom, 1980; Richter and McGaughey, 1981). Evidence for post-translational modification of oocyte proteins during maturation has been presented (Van Blerkom, 1981; see Van Blerkom, 1985). The relative contribution of newly translated proteins from preformed mRNA and of post-translational-modified proteins to the changes in the pattern of proteins during resumption of oocyte maturation remains to be determined.

The electrophoretic pattern of proteins synthesized in rabbit oocyte maturing *in vivo* or *in vitro* was similar (Van Blerkom and McGaughey, 1978). In contrast, different protein patterns were observed when sheep oocytes were matured spontaneously *in vitro* as compared with oocytes triggered by gonadotrophins to mature *in vivo* or *in vitro* in follicles or granulosa cell co-cultures (Warnes *et al.*, 1977; Crosby *et al.*, 1981).

Studies with inhibitors of macromolecule synthesis also demonstrate differences between two groups of mammals. Actinomycin D arrests the maturation of mouse oocytes (Bloom and Mukherjee, 1972; Golbus and Stein, 1976), but this effect is probably

exerted through mechanisms other than inhibition of RNA synthesis (Golbus and Stein, 1976). Indeed, lower concentration of actinomycin does not inhibit GVB in mouse oocytes (Jagiello, 1969; Golbus and Stein, 1976; Crozet and Szöllösi. 1980). In contrast, α-amanitin and cordycepin inhibited GVB in sheep oocytes cultured within their cumulus cells, but did not affect resumption of meiosis in denuded oocytes (Osborn and Moor, 1983a). It seems, therefore, that the effect of RNA synthesis inhibitor on maturation of sheep oocytes is exerted at the cumulus cells.

Inhibition of protein synthesis by puromycin (10–100 μg/ml) or cycloheximide (1 μg/ul) does not prevent spontaneous GVB in cultured mouse oocytes, but meiosis is arrested at the circular bivalent or metaphase I stage (Golbus and Stein, 1976; Schultz and Wasserman, 1977a; Motlik *et al.*, 1986). This result would suggest that GVB in this species is independent of protein synthesis. Nevertheless, experiments employing rat oocytes in which GVB was prevented by culture with dbcAMP and puromycin result in inhibition of maturation when the oocytes are transferred into a medium containing only puromycin (Ekholm and Magnusson, 1979). It seems, therefore, that in mouse and rat oocytes GVB is dependent on the presence of short-lived proteins, which are available in fully grown oocytes and hence GVB can proceed normally even in the presence of protein synthesis inhibitors. In pig oocytes, however, GVB is dependent upon protein synthesis, since cycloheximide (1 μg/ml) prevents GVB in culture (Fulka *et al.*, 1986). Likewise, protein synthesis is required for GVB in ovine and bovine oocytes. Furthermore, synthesis of a polypeptide with M_r47 kDa immediately before GVB was identified in ovine oocytes. Synthesis of this polypeptide was suppressed by inhibition of transcription by α-amanitin (Moor and Crosby, 1986).

On the basis of these observations, it seems that differences exist between mammalian species, such as the sheep and the pig, in which a relatively long interval separates the meiosis-inducing stimulus (gonadotrophin or explantation *in vitro*) and GVB, and species like the mouse, the rat and the rabbit, having a short stimulus–GVB interval. In the latter, puromycin does not block GVB, there is no difference between the pattern of proteins synthesized in the oocytes *in vivo* and *in vitro*, there is no need for undisturbed steroidogenesis during this interval (Moor *et al.*, 1980b), and finally, spontaneously matured oocytes *in vitro* have normal developmental capacity (Schroeder and Eppig, 1984). Conversely, in species with long intervals between the meiosis-inducing stimulus and GVB, full developmental competence is dependent upon follicular environment (Moor and Osborn, 1983), undisturbed steroidogenesis (Moor *et al.*, 1980b; Osborn and Moor, 1983b) and this may be reflected in the difference in the pattern of steroids synthesized in the follicle and in the isolated oocyte (see Thibault *et al.*, 1987). This working hypothesis should be examined systematically in several species.

1.7.2 FOLLICULAR RUPTURE

The follicular response to the surge of gonadotrophins culminates in the rupture of the follicle wall and the release of the fertilizable ovum. Several reviews were published on various aspects of follicular rupture (Rondell, 1970a,b; Lipner, 1973; Espey, 1974; 1978; 1980; LeMaire and Marsh, 1975; Parr, 1975; Wallach *et al.*, 1980; Murdoch, 1985; Thibault and Levasseur, 1988; LeMaire, 1989; Goetz *et al.*, 1991; Tsafriri *et al.*, 1991a). Clearly, multiple mechanisms are involved in the process and many more were considered. Lipner (1988) has reviewed in detail all putative processes suggested to be involved in ovulation. Recently, studies on the role of proteolysis, steroids and of metabolites of arachidonic acid in follicular rupture were undertaken, and these will be reviewed here in brief.

(a) Proteolysis and follicular rupture

The mature preovulatory follicle is embedded within the connective tissue matrix of the ovary, only a small part of which protrudes from the ovarian surface. At its apex the following layers are distinguishable; (i) a single layer of surface epithelium; (ii) collagenous connective tissue consisting of the tunica albuginea and of theca externa – this contributes most of the tensile strength of the follicle wall (Espey, 1967a); (iii) theca interna, a well-vascularized layer containing differentiated fibrocytes very active in steroidogenesis; (iv) granulosa cells, which are separated from the theca interna by the basal lamina and enclose the follicular fluid-filled cavity.

An increase in intrafollicular pressure was for long assumed to be the cause of rupture and extrusion of the oocyte–cumulus complex. Direct measurements of intrafollicular pressure, however, did not reveal any significant increase in rat (Blandau and Rumery, 1963), rabbit (Espey and Lipner, 1963; Rondell, 1964) and pig (Bronson *et al.*, 1979) follicles approaching ovulation. Nevertheless, the preovulatory surge of gonadotrophins is followed by a dramatic increase in the volume of ovulatory follicle(s). Follicular oedema is most probably the result of increased ovarian blood flow (Wurtman, 1964; Blasco *et al.*, 1975; Janson, 1975; Lee and Novy, 1978; Murdoch *et al.*, 1983) and capillary permeability (Lipner and Smith, 1971; Bjersing and Cajander, 1974b; Parr, 1974; Abisogun *et al.*, 1986). In the face of a rapid increase of follicular volume, constant pressure can be maintained only by a concurrent increase in the distensibility of follicular wall. Indeed, as ovulation approaches, a decrease in the tensile strength of follicular wall was observed (Espey, 1967a; Rondell, 1970a,b).

Microscopic examination of the wall of ovulatory follicles revealed marked dissolution of the connective tissue matrix and of collagen fibres in the tunica albuginea and of the theca externa (Espey, 1967b; Bjersing and Cajander, 1974b; see Espey, 1978; Espey *et al.*, 1981). Both the degradative changes and the reduction in follicular tensile strength evidence the pivotal role of proteolytic enzymes in follicular rupture. Involvement of proteolysis in ovulation was suggested already in 1916 by Schochet.

Many enzymes have been identified in follicular tissue or fluid, including proteolytic enzymes (Reichert, 1962; Espey and Rondell, 1967), hyaluronidase (Zachariae and Jensen, 1958), acid phosphatases and esterases (Banon *et al.*, 1964), collagenase-like activity (Espey, 1975; Espey and Coons, 1976; Morales *et al.*, 1978; Fukumoto *et al.*, 1981; Curry *et al.*, 1985; Reich *et al.*, 1985a) and plasminogen activator (PA) (Beers, 1975; Beers *et al.*, 1975; Strickland and Beers, 1976; Canipari and Strickland, 1985; Ny *et al.*, 1985; Reich *et al.*, 1985b, 1986). Furthermore, injection of proteolytic enzymes into the follicular antrum resulted in follicular rupture (Espey and Lipner, 1965), and application of enzyme solutions to follicular wall resulted in ovulatory changes (Espey, 1967a; Espey and Rondell, 1967; Rondell, 1970b).

Studies with preovulatory follicles of mice (Byskov, 1969) and of rabbits (Bjersing and Cajander, 1974c; Cajander, 1976) suggested that the surface epithelium is the source of hydrolytic enzymes which cause ovulation and that dense granules in the cytoplasm of these cells are lysosomes. However, other investigators noticed that ovulation could occur even when the surface epithelium was removed from the follicles (Rawson and Espey, 1977). Another source of proteolytic enzymes are thecal fibroblasts with prominent multivesicular structures, which increase in number following the ovulatory stimulus (Espey, 1971a,b; Espey *et al.*, 1981). The granulosa cells are the major source of follicular PA activity (Reich *et al.*, 1986).

(b) The plasmin activating system and ovulation

The presence of PA was demonstrated in extracts of human and bovine ovarian tissues and in follicular fluid (Albrechtsen, 1957; Beers, 1975; Weimer *et al.*, 1984; Rom *et al.*, 1987). An increase in PA secretion by rat granulosa cells was demonstrated as ovulation approached (Beers *et al.*, 1975; Beers and Strickland, 1978). A preferential increase in PA activity was observed in the apical portion of sow preovulatory follicles (Smokovits *et al.*, 1988). Furthermore, plasmin, the product of PA action on plasminogen, was shown to decrease the tensile strength of Graafian follicle wall (Beers, 1975). All these findings led to the hypothesis that PA is involved in ovulation. It was suggested that plasmin activates latent collagenase and thus initiates the proteolytic changes leading to follicular rupture (Strickland and Beers, 1976; Espey, 1978).

Rat granulosa cell PA activity was stimulated by LH, FSH, cAMP derivatives and prostaglandins E_1 and E_2 (Beers *et al.*, 1975; Strickland and Beers, 1976). In these studies, however, FSH was much more effective than LH in stimulating granulosa cell PA activity (Strickland and Beers, 1976; Martinat and Combarnous, 1983). This is in contrast to the physiological role of LH, and not FSH, as the ovulation-inducing hormone (Schwartz *et al.*, 1973; Tsafriri *et al.*, 1976c). Recent studies seem to resolve this discrepancy. It was found that FSH and LH were equally potent in enhancing rat granulosa cell PA activity provided that the cells were first properly stimulated to induce LH receptors by FSH or dbcAMP (Wang and Leung, 1983). Likewise, FSH, LH, prostaglandin E_1 and relaxin were equally effective in stimulating rat granulosa cell PA, provided that the cells were from mature follicles (Too *et al.*, 1984). Furthermore, in rat pro-oestrous follicles LH and FSH were equally effective in stimulating PA activity (Reich *et al.*, 1985b).

Comparison of *in vitro* accumulation of PA in cultures of rat granulosa cells, whole follicles and the residual tissue, mainly theca with some interstitial tissue (Reich *et al.*, 1986), revealed that the granulosa cells contribute 80–90% of total follicular PA activity. Furthermore, whereas most of granulosa PA activity is secreted into the culture medium, that of intact follicles or residual tissue is retained within the follicle and should be extracted from the tissue (Reich *et al.*, 1985b, 1986). This finding suggests that the theca compartment serves as a specific barrier which prevents the secretion of PA into extrafollicular compartment and, therefore, allows localized action of PA in follicular wall.

Two different molecular types of PA have been identified in mammals, the urokinase type (u-Pa) and the tissue type (t-PA). The two types are products of different genes (Ny *et al.*, 1984; Suenson *et al.*, 1984; Riccio *et al.*, 1985). Two molecular types of PA were identified in rat preovulatory follicles which, on the basis of electrophoretic mobility and immunoabsorption with t-PA antiserum, could be characterized as t-PA and u-PA (Figure 1.11).

The two molecular types were present both in the granulosa and in the theca compartments, the granulosa cells contributing 80–90% of the total follicular activity. Upon gonadotrophin stimulation, a highly significant ($P > 0.001$) increase in t-PA was observed in whole follicles and in the granulosa cells (Reich *et al.*, 1986). Likewise, rat granulosa cells and cumulus–oocyte complexes responded to gonadotrophins by enhanced accumulation of t-PA (Ny *et al.*, 1985; Y.X. Liu *et al.*, 1986). In contrast, measuring only the secreted PA, Canipari and Strickland (1985, 1986) reached the conclusion that the theca (or the whole follicle) secretes u-PA and that this type is stimulated by the gonadotrophin. However, the data of Reich *et al.* (1985b, 1986) indicate that only a minor fraction of follicular PA is secreted into the medium, thus reconciling these apparent discrepancies. A similar

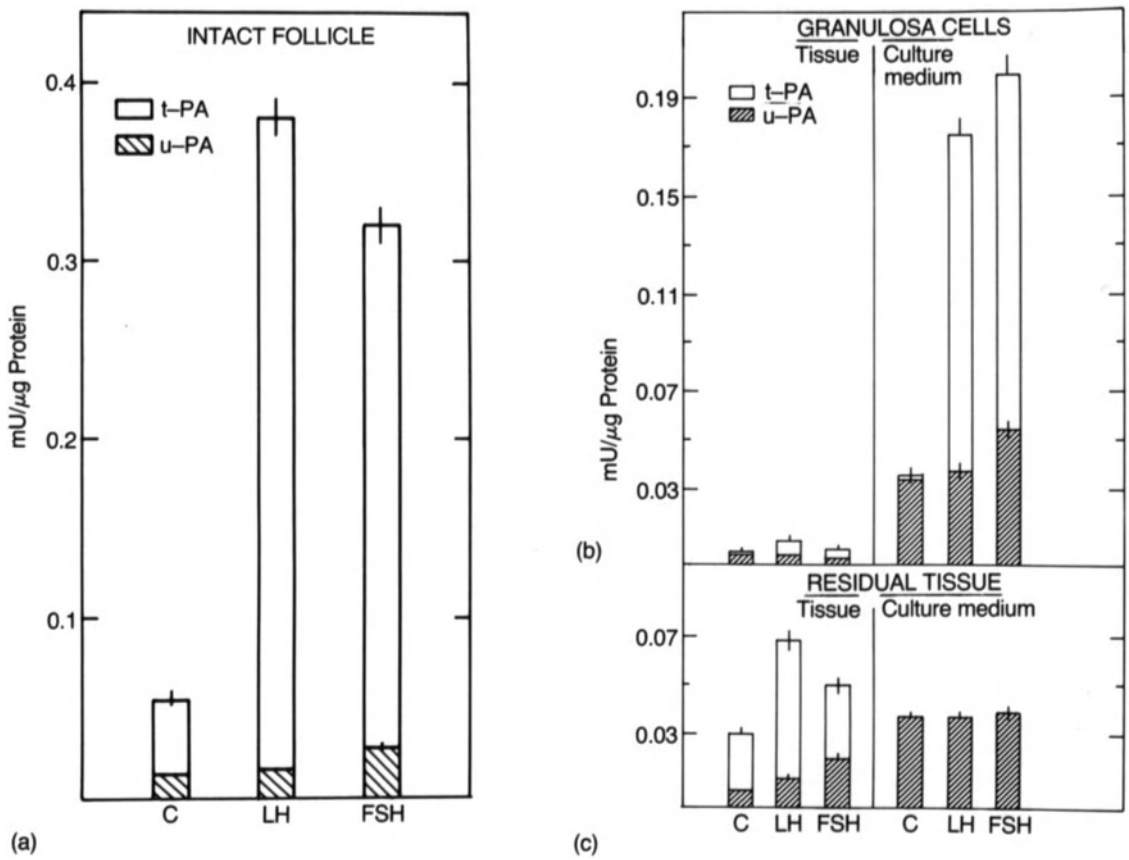

Figure 1.11 Stimulation of follicular PA activity *in vitro* by gonadotrophins (a) in explanted follicles, (b) in cultured granulosa cells and (c) in residual tissue, mainly theca tissue with some interstitial and granulosa cells. PA activity was assayed by quantitative zymographic analysis. (From Reich *et al.*, 1986.)

increase in t-PA in human granulosa cells was observed (Reinthaller *et al.*, 1990). Nevertheless, in mice urokinase seems to be the PA stimulated by the preovulatory surge of gonadotrophins (Canipari *et al.*, 1987).

The involvement of PA in follicular rupture was confirmed by a pharmacological approach. Several inhibitors of serine proteases prevented ovulation in rats *in vivo* (Akazawa *et al.*, 1983a,b; Reich *et al.*, 1985b) and in hamster ovaries *in vitro* (Ichikawa *et al.*, 1983a). Likewise, an antibody to t-PA and a serum-derived inhibitor of plasmin, α_2-antiplasmin (α_2-AP), blocked ovulation when administered to pro-oestrous rats Tsafriri *et al.*, 1989b). The inhibitors of serine proteases anti t-PA and α_2-AP suppressed ovulation effectively only up to 3 h after administration of hCG (Reich *et al.*, 1985b; Tsafriri *et al.*, 1989b) indicating that serine proteases are involved in early changes leading to follicular rupture. Interestingly, suppression of ovulation by anti t-PA and α_2-AP was frequently accompanied by the breakdown of follicular basement membrane and escape of the ovum into the thecal compartment. These results provide the clearest evidence to date for the involvement of t-PA and plasmin in ovulation.

Local PA activity is regulated by specific inhibitors (Hekman and Loskutoff, 1988), plasminogen activator inhibitor type 1 (PAI-1) being the primary physiological inhibitor of both, t-PA and u-PA (Vassali *et al.*, 1991). Granulosa cells secrete PAI-1 and FSH suppressed this activity (Ny *et al.*, 1985). Increased levels of PAI-1 activity were found in human preovulatory follicular fluid (Reinthaller *et al.*, 1990), in ovarian extracellular fluid of hCG-treated immature rats primed with PMSG (Y.X. Liu *et al.*, 1991) and in preovulatory follicles of prepubertal gilts treated with eCG and hCG (Politis *et al.*, 1990). Y.X. Liu *et al.* (1991) using Northern blot analysis demonstrated that the expression of PAI-1 mRNA is regulated by gonadotrophins differentially in granulosa and theca–interstitial cells during the preovulatory period. The expression of PAI-1 mRNA in theca–interstitial cells is maximal at 6 h after LH/hCG stimulation, and in granulosa cells it reaches maximum only 24 h after the stimulation (Y.X. Liu *et al.*, 1991). By *in situ* hybridization only negligible expression of PAI-1 mRNA limited to theca cells was observed before hCG stimulation of ovulation. Following hCG administration, increased expression of PAI-1 mRNA was observed in theca cells of most antral follicles, whereas granulosa cell expression was limited to preovulatory follicles and only to areas where the basal membrane was dissociated (Chun *et al.*, 1992). Glucocorticoids were shown to suppress plasminogen-dependent fibrinogen activity in conditioned medium of a rat hepatoma cell line, and this was later demonstrated to be due to an increase in PAI-1 (Gelehrter *et al.*, 1987; Heaton and Gelehrter, 1990). Curiously, in rat granulosa cells glucocorticoids did not affect PAI-1 activity, but did stimulate PA (Wang and Leung, 1989). This effect of glucocorticoids on granulosa cell t-PA activity probably underlies the observed increase in ovulation rate in the rat after dexamethasone treatment (de Greef and van der Schoot, 1987).

(c) Collagenolysis

The extensive changes in follicular collagen observed prior to ovulation suggest that collagen-degrading enzymes play a central role in ovulation. Collagenase-like activity was demonstrated in ovarian tissues (Morales *et al.*, 1978, 1983; Fukumoto *et al.*, 1981).

Until very recently, experiments to extract follicular collagenase and to demonstrate its ability to degrade follicular wall were not successful (Espey and Stacy, 1970), nor has a correlation between collagenase activity and ovulatory changes been demonstrated (Morales *et al.*, 1978, 1983). This failure should probably be attributed to the fact that collagenase regulation *in vivo* is a multifactorial process involving both cellular and microenvironmental factors. These include cellular factors modulating collagenase proenzyme release and control of its activity by activators, inhibitors and changes in substrate susceptibility. In extracts of rat ovaries an increase in three distinct inhibitors of metalloproteinases, including tissue inhibitor of metalloproteinase (TIMP), was demonstrated after stimulation of ovulation by hCG (Woessner *et al.*, 1989).

In order to circumvent the difficulties in demonstrating gonadotrophin-induced changes in ovarian collagenase activity collagen was labelled by local administration of [^{3}H] proline ([^{3}H] Pro). Some of the [^{3}H] Pro incorporated into collagen molecules is enzymatically hydroxylated into hydroxyproline (Hyp). The presence of the latter is unique to collagen. Ovarian collagen content and collagenolysis were assessed by separating [^{3}H] Hyp from acid hydrolysate of ovarian tissue by high-performance liquid chromatography (Reich *et al.*, 1985b).

Using this approach, significant degradation of labelled collagen was detected after the preovulatory surge of gonadotrophins. Collagen degradation was blocked by Nembutal – a drug which prevents the secretion of gonadotrophins, and hence

ovulation. Moreover, upon administration of hCG on the morning of pro-oestrus, the level of [^{3}H] Hyp in preovulatory follicles was reduced by 43% within 10 h.

Curry *et al.* (1985), employing rigorous extraction procedures and *in vitro* activation of ovarian collagenase by reduction with dithiothreitol, alkylation by iodoacetamide and activation with aminophenylmercuric acetate, confirmed the LH/hCG-induced rise in ovarian collagenase activity. Similar results were obtained by somewhat different extraction procedures, but in these experiments there was no need for collagenase activation *in vitro* (Reich *et al.*, 1987). In view of the difficulties in measuring collagenase activity, examination of ovarian transcription of collagenase mRNA during the periovulatory period may be informative. Indeed, by hybridizing human cDNA of collagenase I with rat ovarian mRNA prepared at various time intervals after triggering ovulation with hCG revealed a stimulation of collagenase mRNA transcription by the hormone, 3–6 h after hCG (Reich *et al.*, 1991).

The role of collagenase in follicular rupture was corroborated indirectly by intrabursal administration of cysteine. It prevented ovarian collagenolysis as well as ovulation, and these two actions were dose related (Reich *et al.*, 1985a). Similarly, a microbial metalloprotease inhibitor, talopeptine, inhibited ovulation *in vitro* from hamster ovaries (Ichikawa *et al.*, 1983b). Finally, a specific inhibitor of mammalian tissue collagenase blocked ovulation in perfused rat ovaries (Brännström *et al.*, 1988) and *in vivo* (D. Daphna-Iken, unpublished observations).

Metalloproteinases, the enzymes including the collagenases, are locally regulated by specific serum and tissue inhibitors. Recently, TIMP has been identified in ovarian tissues (Curry *et al.*, 1989; Woessner *et al.*, 1989) and shown to be induced by gonadotrophins (Curry *et al.*, 1989; Mann *et al.*, 1991; Reich *et al.*, 1991). *In situ* hybridization revealed low constitutive expression of TIMP-1 mRNA in theca cells, which became markedly stimulated within 9 h after hCG stimulation of ovulation in antral and preovulatory follicles. TIMP-1 mRNA expression in granulosa cells was increased by hCG only in preovulatory follicles (Chun *et al.*, 1992).

LH/hCG seem to stimulate ovarian coexpression of both proteolytic enzymes as t-PA and collagenase and their respective inhibitors, PAI-1 and TIMP-1. The preovulatory stimulation of thecal PAI-1 and TIMP-1 even in small antral follicles seems to protect them against the proteolytic activity diffusing from ovulating follicles and thus allows their development in subsequent cycles to the ovulatory stage.

1.7.3 ROLE OF OVARIAN STEROIDS IN FOLLICULAR RUPTURE

Follicular steroidogenesis increases markedly after the preovulatory surge of gonadotrophins. This overall stimulation in steroid production is followed in rat follicles 4–6 h later by an inhibition of androgen and oestrogen synthesis, whereas the secretion of progesterone further increases during the preovulatory period (Lieberman *et al.*, 1975). The involvement of ovarian steroidogenesis in follicular rupture in the rat was suggested by Lipner and Wendelken (1971) and Lipner and Greep (1971), who inhibited follicular rupture by administration of aminoglutethimide and cyanoketone, drugs that block the synthetic pathway to progesterone. Assuming that the failure of ovulation was due to a lack of essential steroid(s) in the presence of the inhibitor, a model suggesting LH stimulation of a steroid which initiates the subsequent events leading to follicular rupture was constructed (Lipner and Greep, 1971). Evidence supporting such a two-step mechanism was presented by Takahashi *et al.* (1974): hypophysectomy of rats at 15:50 h of pro-oestrus resulted in blockage of follicular rupture, and this was effectively reversed by

three consecutive injections of progesterone (5–10 mg each). This concept was challenged by Bullock and Kappauf (1972), who could dissociate the effects of aminoglutethimide and cyanoketone on plasma progesterone and on follicular rupture; thus, they could induce ovulation by hCG when its effect on steroidogenesis was inhibited by aminoglutethimide, and cyanoketone prevented ovulation even when steroid levels in plasma remained unchanged. Nevertheless, it is possible that plasma levels of progesterone are not a precise indicator of follicular levels of steroids, which are probably involved in follicular rupture. The ability of another inhibitor of 3β-hydroxysteroid dehydrogenase, epostane, to inhibit follicular rupture in the rat was demonstrated, and this inhibition was overcome by administration of progesterone (Snyder *et al.*, 1984). Ovulation in the ewe was blocked by an inhibitor of progesterone synthesis (Murdoch *et al.*, 1986). Furthermore, administration of the progesterone receptor antagonist RU 38486 partially blocked ovulation in mature and immature rats in which ovulation was triggered by hCG (Tsafriri *et al.*, 1987). Iwamasa *et al.* (1992) went further and demonstrated that RU 38486 was effective in inhibiting ovulation only when injected up to 4 h after hCG, and this effect was reversed by oxogenous progesterone. The inhibition of ovulation was related to suppression of proteolytic enzymes, most probably metalloproteinases, and their activity was restored by administration of progesterone. This supports the paracrine role of progesterone, exerted through its interaction with cellular receptors, in the mediation of LH/hCG induction of follicular rupture.

Another approach employed was the use of antibodies to steroids. Thus, intrafollicular injections of progesterone antiserum to rabbits blocked ovulation (Swanson and Lipner, 1977). Likewise, administration of progesterone (Mori *et al.*, 1977a) or testosterone (Mori *et al.*, 1977b) antiserum to PMSG–hCG-treated immature rats inhibited follicular rupture and this was overcome by exogenous progesterone, testosterone and 5α-dihydrotestosterone, but not by oestradiol-17β. Thus, *in vivo* studies seem to suggest the role of follicular steroids in follicle rupture.

The role of ovarian steroids was closely examined in the model of perfused ovary which affords study of follicular rupture *in vitro* (Lambertsen *et al.*, 1976; Janson *et al.*, 1982). In perfused rabbit ovaries inhibition of cholesterol chain cleavage enzyme by aminoglutethimide or of 3β-hydroxysteroid dehydrogenase did not affect follicle rupture induced by gonadotrophin (Holmes *et al.*, 1985; Yoshimura *et al.*, 1986). By contrast, an inhibitor of 3β-hydroxysteroid dehydrogenase significantly reduced the ovulation from perfused rat ovaries stimulated by a combination of LH and IBMX (Brännström and Janson, 1989). The preovulatory decline in follicular oestrogen is not required for follicular rupture in the rabbit, since addition of oestrogen (10 μg/ml) to the perfusate did not inhibit ovulation, in spite of a more than twofold increase in follicular oestradiol-17β content (LeMaire *et al.*, 1982). Likewise, blockade of the early rise in oestrogen by an inhibitor of aromatase did not affect follicular rupture in perfused rat ovaries (Koos *et al.*, 1984; Morioka *et al.*, 1988).

While inhibition of steroidogenesis did not affect ovulatory response of perfused ovaries in the rabbit, addition of progesterone into the perfusate modified it. Progesterone (6.7 μg/ml) somewhat enhanced follicular rupture in one study (Hamada *et al.*, 1979), and it reduced the number of ovulating ovaries in another employing a higher dose of progesterone (10 μg/ml) (Holmes *et al.*, 1985). Thus, a modulatory role of progesterone in follicular rupture *in vitro* cannot be excluded. Collectively, the data presented seem to suggest the involvement of steroids, most probably progesterone, in follicular rupture. Nevertheless, their specific role remains obscure and species differences may exist.

Addition of steroids (1 μg/ml) into the medium in which unstimulated preovulatory rat follicles were cultured did not change follicular PA activity during a 6 h incubation period. By contrast, addition of oestradiol-17β, progesterone or testosterone, but not 5α-dihydrotestosterone (all 1 μg/ml) to LH-stimulated follicles further enhanced PA activity. Furthermore, addition of inhibitors of steroidogenesis or of aromatase inhibited the LH-stimulated activity of PA ($P > 0.001$). LH-stimulated follicular PA activity could be restored in the presence of inhibitors by the addition of progesterone, testosterone and oestradiol-17β, but not in the non-aromatizable 5α-dihydrotestosterone (Reich *et al.*, 1985b). More recently, the ability of the antiprogesterone RU 38486 to suppress ovarian proteinases, most probably metalloproteinases, was demonstrated (Iwamasa *et al.*, 1992), thus suggesting a mediatory role for steroids in gonadotrophic stimulation of proteolysis, required for follicle rupture.

1.7.4 ROLE OF EICOSANOIDS

Several studies demonstrated an increase in follicular prostaglandin synthesis after gonadotrophic stimulation (LeMaire *et al.*, 1973; Ainsworth *et al.*, 1975; Armstrong and Zamecnik, 1975; Bauminger and Lindner, 1975). It was shown that this action of LH is not dependent upon steroid synthesis (Bauminger *et al.*, 1975), and that most follicular responses to LH, such as ovum maturation, activation of adenyl cyclase, steroidogenesis and luteinization proceed while prostaglandin synthesis is inhibited by drugs (reviewed by Zor and Lamprecht, 1977). The exception is follicular rupture, which is prevented by administration of indomethacin (rats, Armstrong and Grinwich, 1972; Orczyk and Behrman, 1972; Tsafriri *et al.*, 1972b; rabbits, Grinwich *et al.*, 1972; O'Grady *et al.*, 1972; gilts, Ainsworth *et al.*, 1979: rhesus monkeys, Wallach *et al.*, 1975; marmoset monkeys, Maia *et al.*, 1978; human, Killick and Elstein, 1987), or intrafollicular injection of antiserum to $PGF_{2\alpha}$ in rabbits (Armstrong *et al.*, 1974), or intravenous injection of an antiserum to cyclooxygenase in rats (Satoh *et al.*, 1981). The inhibitory action of indomethacin was overcome by administration of PGE_2 to rats (Tsafriri *et al.*, 1972b) or $PGF_{2\alpha}$ to rhesus monkeys (Wallach *et al.*, 1975).

Furthermore, in the perfused rabbit ovary $PGF_{2\alpha}$ was able to induce follicular rupture by itself or in hCG-indomethacin treated ovaries (Holmes *et al.*, 1983). Collectively, these results suggest that prostaglandins have a physiological role in follicular rupture (see Zor and Lamprecht, 1977; Patrono, 1983; Lipner, 1988).

The recent elucidation of the lipoxygenase pathway of arachidonic acid cascade and availability of specific inhibitors of lipoxygenase led to examination of their role in ovulation. Unilateral administration of lipoxygenase inhibitors into the ovarian bursa of PMSG-stimulated immature or mature rats resulted in a dose-dependent suppression of ovulation, without affecting the contralateral ovary (Reich *et al.*, 1983, 1987). Furthermore, ovarian lipoxygenase activity was demonstrated in homogenates of rat ovaries and follicles (Reich *et al.*, 1985c, 1987) and of human granulosa cells (Feldman *et al.*, 1986). In ovarian tissue of both the rat and the human, products of 5-, 12- and 15-lipoxygenases were identified (Feldman *et al.*, 1986; Reich *et al.*, 1987) and their activity in the rat was stimulated considerably by hCG treatment. These studies suggest the involvement of lipoxygenase products in follicular rupture of ovulation. The scarcity of stable analogue has not yet allowed the identification of the metabolites involved in ovulation. Nevertheless, a specific inhibitor of 5-lipoxygenase, MK 886, suppressed ovulation in immature rats (Tsafriri *et al.*, 1993).

Collectively, both cyclooxygenase and lipoxygenase products of arachidonic acid metabolism seem to be involved in follicular rupture. The finding that specific inhibition

of any of these pathways blocks ovulation further demonstrates the multiple regulation of the process. Arachidonic acid metabolites are involved in a multitude of physiological processes (Piper, 1983; Hayaishi and Yamamoto, 1985), and experimental evidence for their involvement in ovulation through their action on ovarian proteolysis and vasculature is available.

In several studies, doses of indomethacin which inhibited ovulation did not affect LH/hCG-induced increase in PA activity (Shimada *et al.*, 1983; Espey *et al.*, 1985; Reich *et al.*, 1985b). In spite of the fact that higher doses of indomethacin inhibited PA production *in vivo* (Espey *et al.*, 1985) or *in vitro* (Canipari and Strickland, 1986), it seems that the inhibition of prostaglandin synthesis blocks ovulation through mechanism(s) which are not related to PA. Likewise, inhibitors of lipoxygenase which blocked ovulation did not affect follicular PA production (Reich *et al.*, 1985b). This finding does not imply that PA is not involved in follicular rupture, but that increase in PA activity by itself is not a sufficient trigger of ovulation.

Conversely, inhibitors of arachidonic acid metabolism prevented LH/hCG induced collagenolysis in rat ovaries and follicles (Reich *et al.*, 1985b; Tsafriri *et al.*, 1993) and collagenase activity in rabbit ovaries (Kawamura *et al.*, 1986) and ewe follicles (Murdoch *et al.*, 1986). It therefore appears that preovulatory activation of collagenase by LH/hCG is mediated by products of arachidonic acid. It should be noted, however, that the inhibitors of cyclooxygenase or lipoxygenase did not inhibit the activity of ovarian collagenase *in vitro* (Reich *et al.*, 1985b), nor did indomethacin reduce collagenase activity assayed *in vitro* following extraction of and activation (Curry *et al.*, 1986). It seems, then, that while eicosanoids do not affect directly collagenase activity they are involved in the mediation of gonadotrophic activation of the enzyme required for ovulation.

Periovulatory changes in ovarian and follicular microcirculation were described (Janson, 1975; Lee and Novy, 1978; Murdoch *et al.*, 1983). These studies revealed initial increase in blood flow, which in the sheep was followed by a decrease (Murdoch *et al.*, 1983). It is possible that eicosanoids are involved in ovulation by modulating follicular microcirculation. Indeed, in the rabbit, indomethacin reduced the LH-induced hyperaemic response (Lee and Novy, 1978), and in the ewe it prevented the second-phase reduction in follicular blood flow (Murdoch *et al.*, 1983). In the rat, inhibitors of cyclooxygenase and of lipoxygenase prevented the hCG-induced increase in uptake of labelled iodinated bovine serum albumin. By contrast, they did not attenuate the hCG-increased blood flow, as measured by radioactive microspheres (Abisogun *et al.*, 1988b). While the precise mechanisms involved remain to be determined, all these results support the suggested role of eicosanoids in periovulatory changes in ovarian microcirculation and thereby also in ovulation. Thus, eicosanoids are involved in follicular rupture through their modulation of LH/LCG action on ovarian collagen degradation and on microcirculation.

1.7.5 PLATELET-ACTIVATING FACTOR (PAF)

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent bioactive phospholipid. In addition to triggering aggregation and degranulation of platelets, PAF induces margination and activation of neutrophils. Furthermore, PAF is released upon activation of leucocytes. We have demonstrated a preovulatory increase in ovarian and follicular neutrophils, triggered by hCG (Tsafriri *et al.*, 1989c). In view of these changes common to ovulation and inflammation, we have examined the possible involvement of PAF in ovulation. Unilateral injection into the ovarian bursa of a specific PAF antagonist, BN52021, isolated from the Chinese tree *Ginkgo biloba* (Braquet *et al.*, 1985), or its analogue BN52111

resulted in a dose-dependent inhibition of follicle rupture from the treated ovary. The inhibition was prevented when the inhibitors and PAF were administered simultaneously. However, PAF was unable to induce ovulation on its own when administered to rats that were not stimulated by exogenous hCG and had their endogenous surge of LH blocked by LH (Abisogun *et al.*, 1989; Daphna-Iken *et al.*, 1989). A decrease in ovarian PAF was observed after stimulation of ovulation by hCG (Espey *et al.*, 1989).

The PAF antagonist, in addition to inhibiting follicle rupture, suppressed the hCG-stimulated increase in ovarian collagen degradation and vascular permeability, similar to the action of eicosanoid synthesis inhibitors. These results were confirmed in studies employing additional PAF antagonists: in the rat using Y24180 (Li *et al.*, 1991) and in the mouse CV3988 (Kikukawa *et al.*, 1991). PAF was detected in follicular fluid collected from women undergoing *in vitro* fertilization–embryo transfer (Amiel *et al.*, 1991), and the secretion of PAF from ovine follicles *in vitro* was stimulated after the LH surge (Alexander *et al.*, 1990). Conversely, a decrease in rat ovarian PAF was reported after stimulation of ovulation by hCG (Espey *et al.*, 1989). These results support the participation of PAF in follicular rupture. Nevertheless, the precise mechanism of PAF involvement in follicular rupture, as well as its cellular origin and targets in the ovary, remain to be determined.

1.8 CONCLUDING REMARKS

The evolution of the vertebrate ovary shows examples of stable and labile systems (Jones, 1978). This is also clearly demonstrated during the life history of the mammalian female germ cell and its accompanying follicle cells.

Oogenesis in most mammalian species differs from that of several major vertebrate groups in the absence of oogonial mitoses in adult ovaries. By contrast, in teleosts, amphibia and reptiles, oogonia persist in adult ovaries and they continue to divide and differentiate into oocytes. In most vertebrates large crops of oocytes mature simultaneously. In mammals, the evolution of cyclic ovulation of one (in monotocous species) or more (in polytocous species) ova has resulted in hierarchical development of follicles and oocytes. The coexistence of follicles and oocytes at different stages of development in mammals requires mechanisms allowing stage-specific responses to similar endocrine cues. Variability in sensitivity to hormones due to stage-dependent changes in hormone receptors, differential distribution of ovarian microcirculation and hence exposure to varying hormone concentrations, as well as action of locally produced autocrine and paracrine hormones which modulate cellular responses, may all be involved, among other mechanisms, in supporting differential responses. Thus, in mammals during discrete stages of the reproductive cycle, dominant structures, such as follicles or corpora lutea, assume a leading role in ovarian function, rather than the entire ovary. Furthermore, throughout follicular growth a delicately balanced synchronization of the development of the germ cell and its surrounding somatic cells occurs.

Vertebrate egg components are synthesized in the oocyte and in extraoocyte tissues such as the oviduct, liver and, probably, follicle cells (Tate, 1986). The extraoocyte synthesis of its components is clearly coordinated by hormones, hypophyseal gonadotrophins and ovarian oestrogen(s). The role of hormonal signals from endocrine glands or paracrine secretions in the regulation of oocyte autosynthetic activity is, however, less obvious. In oviparous vertebrates, the extraoocyte tissues contribute a relatively small number of specialized products in large amounts that constitute a major fraction of egg mass. In mammals, viviparity eliminated the need for massive contribution of extraoocyte tissues to the oocyte. Whether there

exists any extraoocyte contribution to the mammalian oocyte has yet to be determined.

In fishes and amphibia, pituitary gonadotrophins induce resumption of meiosis through the mediation of follicular (or interrenal) steroids (W.J. Wasserman and Smith, 1978; Schuetz, 1985). By contrast, LH-stimulated steroidogenesis does not seem to mediate the meiosis-inducing action of LH in mammals. Furthermore, the regulation of follicular development and of oocyte maturation seems to be more complicated, including both stimulatory and inhibitory signals by endocrine and paracrine hormones. This increased complexity, as well as the involvement of multiple control mechanisms, is probably associated with hierarchical oocyte/follicle development in mammals. As usual, these multiple regulatory mechanisms utilize hormones which in lower forms serve other functions.

However, only the extraoocyte mechanisms regulating oocyte maturation seem to be divergent in mammals; the molecular changes within the ooplasm, as far as known at the present, seem to be conserved. Thus, MPF from maturing mouse oocytes induced resumption of meiosis when injected into starfish (Kishimoto *et al.*, 1984; H. Hashimoto and Kishimoto, 1988) and toad oocytes (Sorensen *et al.*, 1985). It therefore seems that evolution of the mammalian ovary entails primarily changes in oocyte–follicle interrelationships and in the biochemical mechanisms associated with the follicle control of meiosis. We have discussed the apparent difference between oocytes of various mammals in adenylate cyclase activity and in the resulting dependence on transfer of cyclic AMP from the somatic cells of the follicle.

One of the most striking features of oogenesis is selection. Thus, germ cells are eliminated at various stages of their development. Degeneration afflicts both oogonia and oocytes. Only a limited number of oocytes within primordial follicles initiate their growth during any time interval. Finally, the vast majority of growing follicles undergo atresia rather than ovulation. It seems that rescue from atresia is primarily a matter of synchronization between follicular stage of development and hormonal environment. Only 'follicles being at the right stage and place, at the right time' (Ryan, 1981) are selected and reach ovulation (see Tsafriri and Braw, 1984). Thus, hormonal cues appear to play a major role in atresia. The role of hormones at earlier stages of oocyte degeneration and follicle selection is less obvious. Preprogrammed events are probably also involved in initiation of meiosis and follicular growth and in the development of meiotic competence. The precise interrelationship of preprogrammed changes and hormonal cues in mammalian oogenesis remains to be defined.

Oocytes of the mouse, spontaneously matured *in vitro*, have normal developmental capacity (Schroeder and Eppig, 1984), while those of sheep fail to develop, upon transfer to appropriate recipients. Full developmental potential of sheep and bovine oocytes is dependent upon hormonal stimulation of the intact follicle or co-cultured granulosa cells (Moor *et al.*, 1980b; Moor and Osborn, 1983; Critser *et al.*, 1986). This difference is probably related to the time interval between the meiosis-inducing stimulus and GVB in these species and is reflected as well in their dichotomy regarding the need for undisturbed macromolecular and steroid synthesis during this period. More systematic examination of additional mammalian species is required to test this hypothesis. Clearly, these are only a few of the open questions concerning development of the mammalian ovum, which were alluded to hitherto.

Scientific inquiry is a continuum, and our understanding of ovum development is no exception. In this chapter, we have attempted to present a current account of the life history of the mammalian ovum. Care was taken to represent fairly major advances and divergent approaches and opinions. The

extent of our success is left to the discretion of the reader. We were helped by standing on the shoulders of the giant contributors in this field. It is impossible to mention all their names here, but the seminal contributions of three active scientists who laid the foundations of the current work on mammalian ova deserve special mention, C.R. Austin, M.C. Chang and C. Thibault. Their pioneering studies and insight, we believe, have inspired many of the studies reviewed here.

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REFERENCES

Aberdam, E. and Dekel, N. (1985) Activators of protein kinase C stimulate meiotic maturation of rat oocytes. *Biochemical and Biophysical Research Communications*, **132**, 570–574.

Aberdam, E., Hanski, E. and Dekel, N. (1987) Maintenance of meiotic arrest in isolated rat oocytes by the invasive adenylate cyclase of Bordetella pertussis. *Biology of Reproduction* **36**, 530–535.

Abisogun, A.O., Reich, R., Kranzfelder, D., Rom, E. and Tsafriri, A. (1986) Effect of hCG on perifollicular capillary-network and the uptake of radiolabelled albumin by rat ovaries: Modulation by inhibitors of eicosanoid synthesis. Proceedings of Ovarian Workshop, Ithaca, July 12–14.

Abisogun, A.O., Braquet, P. and Tsafriri, A. (1989) The involvement of platelet activating factor in ovulation. *Science*, **243**, 381–383.

Ainsworth, L., Baker, R.D. and Armstrong, D.T. (1975) Preovulatory changes in follicular fluid prostaglandin F levels in swine. *Prostaglandins*, **9**, 915–925.

Ainsworth, L., Tsang, B.K., Downey, B.R., Baker, R.D., Marcus, G.J. and Armstrong, D.T. (1979) Effects of indomethacin on ovulation and luteal function in gilts. *Biology of Reproduction*, **21**, 401–412.

Akazawa, K., Matsuo, O., Kosugi, T., Mihara, H. and Nori, N. (1983a) The role of plasminogen activator in ovulation. *Acta Physiologica Latino American*, **33**, 105–110.

Akazawa, K., Mori, N., Kosogi, T., Matsou, O. and Mihara, H. (1983b) Localization of fibrinolytic activity in ovulation of the rat follicle as determined by the fibrin slide method. *Japanese Journal of Physiology*, **33**, 1011–1018.

Albertini, D.F. and Anderson, E. (1974) The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. *Journal of Cell Biology*, **63**, 234–250.

Albertini, D.F., Fawcett, D.W. and Olds, P.J. (1975) Morphological variation in gap junctions of ovarian granulosa cells. *Tissue Cell*, **7**, 389–405.

Albrechtsen, O.K. (1957) The fibrinolytic activity of human tissues. *British Journal of Haematology*, **3**, 284–291.

Alexander, B.M., Van Kirk, E.A. and Murdoch, W.J. (1990) Secretion of platelet–activating factor by periovulatory ovine follicles. *Life Sciences*, **47**, 865–868.

Amiel, M.L., Testart, J. and Benveniste, J. (1991) Platelet–activating factor-acether is a component of human follicular fluid. *Fertility and Sterility*, **56**, 62–65.

Amselgrüber, W. (1982) Licht und elektronmidros-

kopische Untersuchungen zur Oogenese der Katze (*Felis Catus*). Dissertation. Munich University.

Amsterdam, A., Koch, Y., Lieberman, M.E. and Lindner, H.R. (1975) Distribution of binding sites for human chorionic gonadotropin in the preovulatory follicle of the rat. *Journal of Cell Biology*, **67**, 894–900.

Amsterdam, A., Josephs, R., Lieberman, M.E. and Lindner, H.R. (1976) Organization of intramembrane particles in freeze-cleaved gap junctions of rat Graafian follicles: Optical-diffraction analysis. *Journal of Cell Science*, **21**, 93–105.

Anand Kumar, T.C. (1968) Oogenesis in the lorises, *Loris tardigradus lydekkerianus* and *Nycticebus concang*. *Proceedings of the Royal Society of London*, Series B, **169**, 167–176.

Andersen, A.C. and Simpson, M.E. (1973) *The Ovary and the Reproductive Cycle of the Dog* (Beagle). Los Altos, CA: Geron-X.

Anderson, E. and Albertini, D.F. (1976) Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *Journal of Cell Biology*, **71**, 680–686.

Anderson, E. and Beams, H.W. (1960) Cytological observations on the fine structure of the guinea pig ovary with special reference to the oogonium, primary oocyte and associate follicle cells. *Journal of Ultrastructural Research*, **3**, 432–446.

Arceo, R.B. and Ryan, K.J. (1967). Conversion of androst-4-ene-3, 17-dione-4-^{14}C to oestrogens by subcellular fractions of the human corpus luteum of the normal cycle. *Acta Endocrinologica*, **56**, 225–230.

Armstrong, D.T. and Grinwich, D.L. (1972) Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins*, **1**, 21–28.

Armstrong, D.T. and Zamecnik, J. (1975) Preovulatory elevation of rat ovarian prostaglandin F and its blockade by indomethacin. *Molecular and Cellular Endocrinology*, **2**, 125–131.

Armstrong, D.T., Grinwich, D.L., Moon, Y.S. and Zamecnik, J. (1974) Inhibition of ovulation in rabbits by intrafollicular injection of indomethacin and PGF_2 antiserum. *Life Sciences*, **14**, 129–140.

Armstrong, D.T., Goff, A.K. and Dorrington, J.H. (1979) Regulation of follicular estrogen biosynthesis in *Ovarian Follicular Development and Function*, (eds A.R. Midgley and W.A. Sadler), Raven Press, New York, pp. 169–181.

Arth, G.E., Patchett, A.A., Jefopoulus, T., Bugianesi, R.L., Peterson, L.H., Ham, E.A., Kuehl, F.A. and Brink, N.G. (1971) Steroidal androgen biosynthesis inhibitors. *Journal of Medical Chemistry*, **14**, 675–679.

Ayalon, D., Tsafriri, A., Lindner, H.R., Cordover, T. and Harrell, A. (1972) Serum gonadotrophin levels in pro-oestrous rats in relation to the resumption of meiosis by the oocytes. *Journal of Reproduction and Fertility*, **31**, 51–58.

Baca, N. and Zamboni, L. (1967) The fine structure of human follicular oocytes. *Journal of Ultrastructural Research*, **19**, 354–381.

Bachvarova, R. (1974) Incorporation of tritiated adenosine into mouse ovum RNA. *Developmental Biology*, **40**, 52–58.

Bachvarova, R. (1981) Synthesis, turnover and stability of heterogeneous RNA in growing mouse oocytes. *Developmental Biology*, **86**, 384–392.

Bachvarova, R. (1985) Gene expression during oogenesis and oocyte development in mammals in *Developmental Biology. A Comprehensive Synthesis*, Vol. 1, (ed. L.W. Browder), Oogenesis, Plenum Press, New York, pp. 453–524.

Bachvarova, R., Baran, M.M. and Tejblum, A. (1980) Development of naked growing mouse oocytes *in vitro*. *Journal of Experimental Zoology*, **211**, 159–169.

Bachvarova, R., De Leon, V., Johnson, A., Kaplan, G. and Paynton, B.V. (1985) Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. *Developmental Biology*, **208**, 325–331.

Baird, D.T. (1977) Evidence *in vivo* for the two-cell hypothesis of oestrogen synthesis by the sheep Graafian follicle. *Journal of Reproduction and Fertility*, **50**, 183–185.

Baird, D.T. (1983) Factors regulating the growth of the pre-ovulatory follicle in the sheep and human. *Journal of Reproduction and Fertility*, **69**, 343–352.

Baird, D.T. (1987) A model for follicular selection and ovulation: Lessons from superovulation. *The Journal of Steroid Biochemistry*, **27**, 15–23.

Baird, D.T. and McNeilly, A.S. (1981) Gonadotrophic control of follicular development and function during the oestrous cycle of the ewe. *Journal of Reproduction and Fertility*, Suppl. 30, 119–133.

Baird, D.T., Baker, T.G., McNatty, K.P. and Neal, P. (1975). Relationship between the secretion of the corpus luteum and the length of the follicu-

lar phase of the ovarian cycle. *Journal of Reproduction and Fertility*, **45**, 611–619.

Baird, D.T., Swanston, I.A. and McNeilly, A.S. (1981) Relationship between LH, FSH and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe. *Biology of Reproduction*, **24**, 1013–1025.

Baird, D.T., Bäckström, T., McNeilly, A.S., Smith, S.K. and Wathen, C.G. (1984) Effect of enucleation of the corpus luteum at different stages of the luteal phase of the human menstrual cycle on subsequent follicular development. *Journal of Reproduction and Fertility*, **70**, 615–624.

Baker, T.G. (1963) A quantitative and cytological study of germ cells in the human ovaries. *Proceedings of the Royal Society of London*, Series B, **158**, 416–433.

Baker, T.G. (1966) A quantitative and cytological study of oogenesis in the rhesus monkey. *Journal of Anatomy*, **100**, 761–776.

Baker, T.G. (1972) Oogenesis and ovarian development in *Reproductive Biology*, (eds H. Balin and S. Glasser), Excerpta Medica, Amsterdam, pp. 298–437.

Baker, T.G. (1982) Oogenesis and ovulation in *Reproduction in mammals. 1 Germ Cells and fertilization*, 2nd edn, (eds C.R. Austin and R.V. Short), Cambridge University Press, pp. 17–45.

Baker, T.G. and Franchi, L.L. (1967a) The fine structure of oogonia and oocytes in human ovaries. *Journal of Cell Science*, **2**, 213–224.

Baker, T.G. and Franchi, L.L. (1967b) The structure of the chromosomes in human primordial oocytes. *Chromosoma*, **22**, 358–377.

Baker, T.G. and Franchi, L.L. (1972) The fine structure of oogonia and oocytes in the Rhesus monkey (*Macaca mulatta*). *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **126**, 53–74.

Baker, T.G. and O, W.S. (1976) Development of the ovary and oogenesis. *Clinical Obstetrics and Gynaecology*, **3**, 3–26.

Baker, T.G. and Scrimgeour, J.B. (1980) Development of the gonad in normal anencephalic human fetuses. *Journal of Reproduction and Fertility*, **60**, 193–199.

Baker, T.G., Beaumont, M.H. and Franchi, L.L.(1969). The uptake of tritiated uridine and phenylalanine by the ovaries of rats and monkeys. *Journal of Cell Science*, **4**, 655–675.

Bakken, A.H. and McClanahan, M. (1978) Patterns of RNA synthesis in early meiotic prophase oocytes from fetal mouse ovaries. *Chromosoma*, **67**, 21–40.

Balakier, H. (1978) Induction of maturation in small oocytes from sexually immature mice by fusion with meiotic or mitotic cells. *Experimental Cell Research*, **112**, 137–141.

Ball, J.H. and Kadis, B. (1965) Steroid hydroxylations. II. Intracellular location of 17α-hydroxylase and its substrate specificity in sow ovary. *Archives of Biochemistry and Biophysics*, **110**, 427–431.

Banon, P., Brandes, D. and Frost, J.K. (1964) Lysosomal enzymes in the rat ovary and endometrium during the estrous cycle. *Acta Cytologica*, **8**, 416–425.

Bar-Ami, S. and Tsafriri, A. (1981) Acquisition of meiotic competence in the rat: Role of gonadotropin and estrogen. *Gamete Research*, **4**, 463–472.

Bar-Ami, S. and Tsafriri, A. (1986) The development of meiotic competence in the rat: Role of hormones and of the stage of follicular development. *Gamete Research*, **13**, 39–46.

Bar-Ami, S., Nimrod, A., Brodie, A.M.H. and Tsafriri, A. (1983) Role of FSH and oestradiol 17-β in the development of meiotic competence in rat oocytes. *Journal of Steroid Biochemistry*, **19**, 965–971.

Baranska, W., Konwinski, M. and Kujawa, M. (1975) Fine structure of the zona pellucida of unfertilized egg cells and embryos. *Journal of Experimental Zoology*, **192**, 193–202.

Baulieu, E.-E. and Schorderet-Slatkine, S. (1983) Steroid and peptide control mechanisms in membrane of Xenopus laevis oocytes resuming meiotic division in *Molecular Biology of Egg Maturation*, (eds R. Porter and J. Whelan), Ciba Foundation Symposium 98, Pitman, London, pp. 137–170.

Bauminger, S. and Lindner, H.R. (1975) Periovulatory changes in ovarian prostaglandin formation and their hormonal control in the rat. *Prostaglandins*, **9**, 737–751.

Bauminger, S., Lieberman, M.E. and Lindner, H.R. (1975) Steroid-independent effect of gonadotropins on prostaglandin synthesis in rat Graafian follicles *in vitro*. *Prostaglandins*, **9**, 753–764.

Beaumont, H.M. (1966) The effects of acute X-irradiation on primordial germ cells in the female rat. *International Journal of Radiation and Biology*, **10**, 17–28.

Beaumont, H.M. and Mandl, A.M. (1962) A quantitative and cytological study of oogonia and

oocytes in the foetal and neonatal rat. *Proceedings of the Royal Society of London*, Series B, **155**, 557–579.

Beers, W.H. (1975) Follicular plasminogen and plasminogen activator and the effect of plasmin on ovarian follicular wall. *Cell*, **6**, 379–386.

Beers, W.H. and Strickland, S. (1978) A cell culture assay for follicle-stimulating hormone. *Journal of Biological Chemistry*, **253**, 3877–3881.

Beers, W.H., Strickland, S. and Reich, E. (1975) Ovarian plasminogen activator: Relationship to ovulation and hormonal regulation. *Cell*, **6**, 387–394.

Bennet, D. (1956) Developmental analysis of a mutant with pleiotropic effects in the mouse. *Journal of Morphology*, **98**, 199–234.

Biggers, J.D. (1971). Metabolism of mouse embryos. *Journal of Reproduction and Fertility*, Suppl. 14, 41–54.

Biggers, J.D. (1972) *Metabolism of the Oocyte*, (eds J.D. Biggers and A.W. Schuetz), *Oogenesis*, University Park Press, Baltimore, pp. 241–251.

Biggers, J.D., Whittingham, D.G. and Donahue, R.P. (1967) The pattern of energy metabolism in the mouse oocyte and zygote. *Proceedings of the National Academy of Sciences of the USA*, **58**, 560–567.

Billig, H., Hedin, L. and Magnusson, C. (1983) Gonadotrophins stimulate lactate production by rat cumulus and granulosa cells. *Acta Endocrinologica*, **103**, 562–566.

Billig, M., Bränström, M., Törnell, J. and Magnusson, C. (1985) Guanosine and hypoxanthine inhibit rat oocyte maturation. *Acta Physiologica Scandinavica*, **123**, Suppl.12, Abstract C12.

Bjersing, L. (1978) Maturation, morphology and endocrine function of the follicular wall in mammals in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 181–214.

Bjersing, L. and Cajander, S. (1974a) Ovulation and the mechanism of follicular rupture. IV. Ultrastructure of membrana granulosa of rabbit Graafian follicles prior to induced ovulation. *Cell and Tissue Research*, **153**, 1–14.

Bjersing, L. and Cajander, S. (1974b) Ovulation and the mechanism of follicular rupture. VI. Ultrastructure of theca interna and the inner vascular network surrounding rabbit Graafian follicles prior to induced ovulation. *Cell and Tissue Research*, **153**, 31–44.

Bjersing, L. and Cajander S. (1974c) Ovulation and the mechanism of follicle rupture. III. Transmission electronic microscopy of rabbit germinal epithelium prior to induced ovulation. *Cell and Tissue Research*, **149**, 313–327.

Black, J.L. and Erickson, B.H. (1968) Oogenesis and ovarian development in the prenatal pig. *Anatomical Record*, **161**, 45–55.

Blandau, R.J. and Rumery, R.E. (1963) Measurements of intrafollicular pressure in ovulatory and preovulatory follicles of the rat. *Fertility and Sterility*, **14**, 330–341.

Blasco, L., Wu, C.H., Flickinger, G.L., Pearlmutter, D. and Mikhail, G. (1975) Cardiac output and genital blood flow distribution during the preovulatory period in rabbits. *Biology of Reproduction*, **13**, 581–586.

Bleil, J.D. and Wassarman, P.M. (1980a) Structure and function of the zona pellucida: Identification and characterization of the proteins of the mouse oocyte's zona pellucida. *Developmental Biology*, **76**, 185–202.

Bleil, J.D. and Wassarman, P.M. (1980b) Mammalian sperm–egg interactions: Identification of a glycoprotein in mouse egg zonae pellucidae possessing receptor activity for sperm. *Cell*, **20**, 873–882.

Bleil, J.D. and Wassarman, P.M. (1980c) Synthesis of zona pellucida proteins by denuded and follicle-enclosed mouse oocytes during culture *in vitro*. *Proceedings of the National Academy of Sciences of the USA*, 77, 1029–1033.

Bleil, J.D. and Wassarman, P.M. (1983) Sperm–egg interactions in the mouse: Sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Developmental Biology*, **95**, 317–324.

Bloom, A.M. and Mukherjee, B.B. (1972) RNA synthesis in maturing mouse oocytes. *Experimental Cell Research*, **74**, 577–582.

Bogovich, K, Richards, J.S. and Reichert Jr, L.E. (1981) Obligatory role of luteinizing hormone in the initiation of preovulatory follicular growth in the pregnant rat: specific effects of human chorionic gonadotropin and follicle-stimulating hormone on LH receptors and steroidogenesis in theca, granulosa and luteal cells. *Endocrinology*, **109**, 860–867.

Bornslaeger, E.A. and Schultz, R.M. (1985) Regulation of mouse oocyte maturation: Effect of elevating cumulus cell cAMP on oocyte cAMP levels. *Biology of Reproduction*, **33**, 698–704.

Bornslaeger, E.A., Wilde, M.W. and Schultz, R.M. (1984) Regulation of mouse oocyte maturation: Involvement of cyclic AMP phosphodiesterase

and calmodulin. *Developmental Biology*, **105**, 488–499.

Bornslaeger, E.A., Poneymirou, W.T., Mattei, P. and Schultz, R.M. (1986) Effects of protein kinase C activators on germinal vesicle breakdown and polar body emission of mouse oocytes. *Experimental Cell Research*, **165**, 507–517.

Bornslaeger, E.A., Mattel, P.M. and Schultz, R.M. (1988) Protein phosphorylation in meiotically competent and incompetent mouse oocytes. *Molecular Reproduction and Development*, **1**, 19–25.

Borum, K. (1961) Oogenesis in the mouse. A study of the meiotic prophase. *Experimental Cell Research*, **24**, 495–507.

Borum, K. (1966) Oogenesis in the mouse. A study of the origin of the mature ova. *Experimental Cell Research*, **45**, 39–47.

Bousquet, D., Léveillé, M.C., Roberts, K.D., Chapdelaine, A. and Bleau, G. (1981) The cellular origin of the zona pellucida antigen in the human and hamster. *Journal of Experimental Zoology*, **215**, 215–218.

Brambell, F.W.R. (1928) The development and morphology of the gonads of the mouse. III The growth of the follicles. *Proceedings of The Royal Society of London*, Series B, **103**, 258–272.

Brambell, F.W.R. (1956). Ovarian changes in *Marshall's Physiology of Reproduction*, Vol. 1, part 1, (ed. A.S. Parkes), Longmans, Green & Co, New York, pp. 397–542.

Brännström, M. and Janson, P.O. (1989) Progesterone is a mediator in the ovulatory process of the *in vitro*-perfused rat ovary. *Biology of Reproduction*, **40**, 1170–1178.

Brännström, M., Woessner Jr, J.F., Koos, R.D., Sear, C.H.J. and LeMaire, W.J. (1988) Inhibitors of mammalian tissue collagenase and metalloproteinases suppress ovulation in the perfused rat ovary. *Endocrinology*, **122**, 1715–1721.

Braquet, P., Spinnewyn, B., Braquet, M., Bourgain, R.M., Taylor, J.E., Etienne, A. and Drien, K. (1985) BN52021 and related compounds: a new series of highly specific PAF-acether receptor antagonists isolated from *Ginkgo biloba*. *Blood Vessels*, **16**, 559–572.

Braw, H.R. and Tsafriri, A. (1980) Effect of PMSG on follicular atresia in the immature rat ovary. *Journal of Reproduction and Fertility*, **59**, 267–272.

Brinster, R.L. (1967) Protein content of the mouse embryo during the first five days of development. *Journal of Reproduction and Fertility*, **13**, 413–420.

Brodie, A.M.H., Schwarzel, W.C., Shaikh, A.A. and Brodie, H.J. (1977) The effect of an aromatase inhibitor, 4-hydroxy-4-androstene-3,17-dione on estrogen-dependent processes in reproduction and breast cancer. *Endocrinology*, **100**, 1684–1695.

Bronson, R.A., Bryant, G., Balk, M.W. and Emanuele, N. (1979) Intrafollicular pressure within preovulatory follicles of the pig. *Fertility and Sterility*, **31**, 205–213.

Brower, P.T. and Schultz, R.M. (1982) Intercellular communication between granulosa cells and mouse oocytes: Existence and possible nutritional role during oocyte growth. *Developmental Biology*, **90**, 144–153.

Brower, P.T., Gizang, E., Boreen, S. and Schultz, R.M. (1981) Biochemical studies of mammalian oogenesis: Synthesis and stability of various classes of RNA during growth of the mouse oocyte *in vitro*. *Developmental Biology*, **86**, 373–383.

Brown, J.B. (1978) Pituitary control of ovarian function – concept derived from gonadotrophin therapy. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, **18**, 47–54.

Bullock, D.W. and Kappauf, B.H. (1972) Dissociation of gonadotropin-induced ovulation and steroidogenesis in immature rats. *Endocrinology*, **92**, 1625–1628.

Burgoyne, P.S. and Baker, T.G. (1981) Oocyte depletion in XO mice and their XX sibs from 12 to 200 days *post partum*. *Journal of Reproduction and Fertility*, **61**, 207–212.

Burkholder, G.D., Comings, D.E. and Okada, T.A. (1971) A storage form of ribosomes in mouse oocytes. *Experimental Cell Research*, **69**, 361–371.

Buscaglia, M., Fuller, J., Mazzola, T., Bacigalupi, K., Shimasaki, S., Ui, M., Simmons, D., Castillo, F., Schroeder, R. and Ling, N. (1989) A new intraovarian function for follistatin: the inhibition of oocyte meiosis. *Proceedings 71st Annual Meeting of the Endocrine Society, Seattle, Washington*, The Endocrine Society, Bethesda, Maryland, pp. 243, abstract 883.

Byskov, A.G. (1969) Ultrastructural studies in the preovulatory follicle in the mouse ovary. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **10**, 285–299.

Byskov, A.G. (1974) Does the rete ovarii act as a trigger for the onset of meiosis? *Nature*, **252**, 396–397.

Byskov, A.G. (1975) The role of the rete ovarii in meiosis and follicle formation in the cat, mink

and ferret. *Journal of Reproduction and Fertility*, **45**, 201–209.

Byskov, A.G. (1978a) The meiosis inducing interaction between germ cells and rete cells in the fetal mouse gonad. *Annales de Biologie Animale, Biochimie et Biophysique*, **18**, 327–334.

Byskov, A.G. (1978b) Follicular atresia in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Publishing Corporation, New York, pp. 533–562.

Byskov, A.G. (1986) Differentiation of mammalian embryonic gonad. *Physiological Reviews*, **66**, 71–117.

Byskov, A.G. and Høyer, P.E. (1987) Embryology of mammalian gonads and ducts in *The Physiology of Reproduction*, Vol. I (eds E. Knobil and J.D. Neill), Raven Press, New York, pp. 265–302.

Byskov, A.G. and Lintern-Moore S. (1973) Follicle formation in the immature mouse ovary: the role of the rete ovarii. *Journal of Anatomy*, **116**, 207–217.

Byskov, A.G. and Rasmussen, G. (1973) Ultrastructural studies of the developing follicle in *The Development and Maturation of the Ovary and Its Function*, (ed. H. Peters), Excerpta Medica, Amsterdam, pp. 55–62.

Byskov, A.G. and Saxén, L. (1976) Induction of meiosis in fetal mouse testis *in vitro*. *Developmental Biology*, **52**, 193–200.

Byskov, A.G., Skakkebaek, N.E., Stafanger, G. and Peters H. (1977) Influence of ovarian surface epithelium and rete ovarii on follicle formation. *Journal of Anatomy*, **123**, 77–86.

Cahill, L.P. and Mauléon, P. (1980) Influences of season, cycle and breed on follicular growth rates in sheep. *Journal of Reproduction and Fertility*, **58**, 321–328.

Cahill, L.P., Mariana, J.C. and Mauléon, P. (1979) Total follicular populations in ewes of high and low ovulation rates. *Journal of Reproduction and Fertility*, **55**, 27–36.

Cahill, L.P., Draincourt, M.A., Chamley, W.A. and Findlay, J.K. (1985a) Role of intrafollicular regulators and FSH in growth and development of large antral follicles in sheep. *Journal of Reproduction and Fertility*, **75**, 599–607.

Cahill, L.P., Clarke, I.J., Cummins, J.T., Draincourt, M.A., Carson, R.S. and Findlay, J.K. (1985b). An inhibitory action at the ovarian level of ovine follicular fluid on PMSG-induced folliculogenesis in hypophysectomized ewes, in *Proceedings of the Fifth Ovarian Workshop* (eds D.O. Toft and R.J. Ryan), Ovarian Workshops, Champaign, pp. 35–38.

Cajander, S. (1976) Structural alteration of rabbit ovarian follicles after mating with special reference to the overlying surface epithelium. *Cell and Tissue Research*, **173**, 437–499.

Cameron, I.L., Lum, J.B., Nations, C., Asch, R.H. and Silverman, A.Y. (1983) Assay for characterization of human follicular oocyte maturation inhibitor using Xenopus oocytes. *Biology of Reproduction*, **28**, 817–822.

Canipari, R. and Strickland, S. (1985) Plasminogen activator in the rat ovary. Production and gonadotropin regulation of the enzyme in granulosa thecal cells. *Journal of Biological Chemistry*, **260**, 5121–5125.

Canipari, R. and Strickland, S. (1986) Studies on the hormonal regulation of plasminogen activator production in the rat ovary. *Endocrinology*, **118**, 1652–1659.

Canipari, R., Pietrolucci, A. and Mangia, F. (1979) Increase of total protein synthesis during mouse oocyte growth. *Journal of Reproduction and Fertility*, **57**, 405–413.

Canipari, R., Palombi, F., Riminucci, M. and Mangia, F. (1984) Early programming of maturation of competence in mouse oogenesis. *Developmental Biology*, **102**, 519–524.

Canipari, R., O'Connell, M.L., Meyer, G. and Strickland, S. (1987) Mouse ovarian granulosa cells produce urokinase-type plasminogen activator, whereas the corresponding rat cells produce tissue-type plasminogen activator. *Journal of Cell Biology*, **205**, 977–981.

Casico, S.M. and Wassarman, P.M. (1981) Programme of early development in the mammal: Synthesis of mitochondrial proteins during oogenesis and early embryogenesis in the mouse. *Developmental Biology*, **83**, 166–172.

Casico, S.M. and Wassarman, P.M. (1982) Program of early development in the mammal: Post-transcriptional control of a class of proteins synthesized by mouse oocytes and early embryos. *Developmental Biology*, **89**, 397–408.

Cattanach, B.M., Iddon, C.A., Charlton, H.M., Chiappa, S.A. and Fink, G. (1977) Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism. *Nature*, **269**, 338–340.

Centola, G.M., Anderson, L.D. and Channing, C.P. (1981) Oocyte maturation inhibitor (OMI) activity in porcine granulosa cells. *Gamete Research*, **4**, 451–461.

Chang, M.C. (1955) The maturation of rabbit oocytes in culture and their maturation, activation, fertilization and subsequent development in Fallopian tube. *Journal of Experimental Zoology*, **128**, 378–399.

Channing, C.P. (1969) Steroidogenesis and morphology of human ovarian cell types in tissue culture. *Journal of Endocrinology*, **45**, 297–308.

Channing, C.P. (1980) Progesterone and estrogen secretion by cultured monkey ovarian cell types: Influences of follicular size, serum luteinizing hormone levels, and follicular fluid oestrogen levels. *Endocrinology*, **107**, 342–352.

Channing, C.P. and Coudert, S.P. (1976) Contribution of granulosa cells and follicular fluid to ovarian estrogen secretion in the rhesus monkey *in vivo*. *Endocrinology*, **98**, 590–597.

Channing, C.P. and Kammerman, S. (1973) Characteristics of gonadotropin receptors of porcine granulosa cells during follicle maturation. *Endocrinology*, **92**, 531–540.

Channing, C.P. and Tsafriri, A. (1977) Mechanism of action of luteinizing hormone and follicle stimulating hormone in the ovary *in vitro*. *Metabolism*, **26**, 413–468.

Channing, C.P., Bae, I.H., Stone, S.L., Anderson, L.D., Edelson, S. and Fowler, S.C. (1981) Porcine granulosa and cumulus cell properties: LH/hCG-receptors, ability to secrete progesterone and ability to respond to LH. *Molecular and Cellular Endocrinology*, **22**, 359–370.

Channing, C.P., Anderson, L.D., Hoover, D.J., Kolena, J., Osteen, K.G., Pomerantz, S.H. and Tanabe, K. (1982) The role of non-steroidal regulators in control of oocyte and follicular maturation. *Recent Progress in Hormone Research*, **38**, 331–408.

Channing, C.P., Liu, C-Q., Jones, G.S. and Jones, H. (1983) Decline in follicular oocyte maturation inhibitor coincident with maturation and achievement of fertilizability of oocytes recovered at midcyle of gonadotropin-treated women. *Proceedings of the National Academy of Sciences of the USA*, **80**, 4184–4188.

Channing, C.P., Gagliano, P., Tanabe, K., Fortuny, A. and Cortes-Prieto, J. (1985) Demonstration of a gradient in inhibin activity, estrogen, progesterone, and δ^4-androstenedione in follicular fluid, ovarian vein blood and peripheral blood of normal women. *Fertility and Sterility*, **43**, 142–145.

Chari, S., Hillensjö, T., Magnusson, C., Sturm, G. and Daume, E. (1983) *In vitro* inhibition of rat oocyte meiosis by human follicular fluid fractions. *Archives of Gynaecology*, **233**, 155–164.

Chiquoine, A.D. (1954) The identification, origin and migration of the primordial germ cells in the mouse embryo. *Anatomical Record*, **118**, 135–145.

Chiras, D.D. and Greenwald, G.S. (1977) An autoradiographic study of long-term follicular development in the cyclic hamster. *Anatomical Record*, **188**, 331–337.

Cho, W.K., Stern, S. and Biggers, J.D. (1974) Inhibitory effect of dibutyryl cAMP on mouse oocyte maturation *in vitro*. *Journal of Experimental Zoology*, **187**, 383–386.

Chretien, F.C. (1966) Étude de l'origine, de la migration et de la multiplication des cellules germinales chez l'embryon de lapin. *Journal of Embryology and Experimental Morphology*, **16**, 591–607.

Chun, S.Y., Popliker, M., Reich, R. and Tsafriri, A. (1992) Localization of preovulatory expression of plasminogen activator inhibitor type-1 and tissue inhibitor of metalloproteinase type-1 in the rat ovary. *Biology of Reproduction*, **47**, 245–253.

Chung, K.W.A. (1974) A morphological and histochemical study of Sertoli cells in normal and XX sex-reversed mice. *American Journal of Anatomy*, **139**, 369–388.

Clark, J.M. and Eddy, E.M. (1975) Fine structural observation on the origin and associations of primordial germ cells of the mouse. *Developmental Biology*, **47**, 136–155.

Clark, M.R., Thibier, C., Marsh, J.M. and LeMaire, W.J. (1980) Stimulation of prostaglandin accumulation by luteinizing hormone-releasing hormone (LHRH) and LHRH analogs in rat granulosa cells *in vitro*. *Endocrinology*, **107**, 17–23.

Colonna, R. and Mangia, F. (1983) Mechanisms of amino acid uptake in cumulus-enclosed mouse oocytes. *Biology of Reproduction*, **28**, 797–803.

Comings, D.E. and Okada, T.A. (1972) Architecture or meiotic cells and mechanisms of chromosome pairing. *Advances in Cellular and Molecular Biology*, **2**, 310–384.

Critser, E.S., Leibfried-Rutledge, M.L., Eyestone, W.H., Northey, D.L. and First, N.L. (1986) Acquisition of developmental competence during maturation *in vitro*. *Theriogenology*, **25**, 150.

Crone, M., Levy, E. and Peters, H. (1965) The duration of the premeiotic DNA synthesis in mouse oocytes. *Experimental Cell Research*, **39**, 678–688.

Crosby, I.M., Osborn, J.C. and Moor, R.M. (1981) Follicle cell regulation of protein synthesis and developmental competence in sheep oocytes. *Journal of Reproduction and Fertility*, **62**, 575–582.

Crosby, I.M., Osborn, J.C. and Moor, R.M. (1984) Changes in protein phosphorylation during the maturation of mammalian oocytes *in vitro*. *Journal of Experimental Zoology*, **229**, 459–466.

Cross, P.C. and Brinster, R.L. (1974) Leucine uptake and incorporation at three stages of mouse oocyte maturation. *Experimental Cell Research*, **86**, 43–46.

Crozet, N. and Szöllösi, D. (1980) Effects of actinomycin D and α-amanitin on the nuclear ultrastructure of mouse oocytes. *Biology Cellulaire*, **38**, 163–170.

Curry, T.E., Dean, D.D., Woessner, J.F. and LeMaire, W.J. (1985) The extraction of a tissue collagenase associated with ovulation in the rat. *Biology of Reproduction*, **33**, 981–991.

Curry, T.E. Jr., Clark, M.R., Dean, D.D., Woessner, J.F.Jr. and LeMaire, W.J.Jr. (1986) The preovulatory increase in ovarian collagenase activity in the rat is independent of prostaglandin production. *Endocrinology*, **118**, 1823–1828.

Curry, T.E. Jr., Dean, D.D., Snaders, S.L., Pedigo, N.G. and Jones, P.B.C. (1989) The role of ovarian proteases and their inhibitors in ovulation. *Steroids*, **54**, 501–521.

Daphna-Iken, D., Chun, S.Y., Abisogun, A.O. and Tsafriri, A. (1989) Platelet activating factor, ginkgolides and follicle rupture during ovulation in *Ginkgolides – Chemistry, Biology, Pharmacology and Clinical Perspectives*, Vol. 2 (ed. P. Bracquet), J.R. Prous Science Publishers, Barcelona, Spain, pp. 835–844.

David, G.F.X., Arnand Kumar, T.C. and Baker, T.G. (1974) Uptake of tritiated thymidine by primordial germinal cells in the ovaries of the adult slender loris. *Journal of Reproduction and Fertility*, **41**, 447–451.

Deanesly, R. (1970) Oogenesis and the development of the ovarian interstitial tissue in the ferret. *Journal of Anatomy*, **107**, 165–178.

De Felici, M. and Siracusa, G. (1985) Adhesiveness of mouse primordial germ cells to follicular and Sertoli cell monolayers. *Journal of Embryology and Experimental Morphology*, **87**, 87–97.

de Greef, W.J. and van der Schoot, P. (1987) Effects of dexamethasome on ovarian activity in the rat. *Acta Endocrinologica*, **116**, 350–356.

Dekel, N. (1986) Hormonal control of ovulation in *Biochemical Actions of Hormones*, Vol. 13 (ed. G. Litwack), Academic Press, New York, pp. 57–90.

Dekel, N. (1987) Interaction between the oocyte and the granulosa cells in the preovulatory follicle in *Endocrinology and Physiology of Reproduction*, (eds P.C.K. Leung, D.T. Armstrong, K.B. Ruf, W.H. Moger and H.G. Friesen), Plenum Press, New York, pp. 197–209.

Dekel, N. and Beers, W.H. (1978) Rat oocyte maturation *in vitro*: relief of cyclic AMP inhibition by gonadotropins. *Proceedings of the National Academy of Sciences of the USA*, **75**, 3469–3473.

Dekel, N. and Beers, W.H. (1980) Development of rat oocyte *in vitro*: inhibition and induction of maturation in the presence or absence of the cumulus oophorus. *Developmental Biology*, **75**, 247–252.

Dekel, N. and Kraicer, P.F. (1978) Induction *in vitro* of mucification of rat cumulus oophorus by gonadotrophins and adenosine 3',5'-monophosphate. *Endocrinology*, **102**, 1797–1802.

Dekel, N. and Piontkewitz, Y. (1991) Induction of maturation of rat oocyte by interruption of communication in the cumulus oocyte complex. *Bulletin des Anatomistes*, **75**, 51–53.

Dekel, N. and Sherizly, I. (1983) Induction of maturation in rat follicle-enclosed oocyte by forskolin. *FEBS Letters*, **151**, 153–155.

Dekel, N. and Sherizly, I. (1985) Epidermal growth factor induces maturation of rat follicle-enclosed oocytes. *Endocrinology*, **116**, 406–409.

Dekel, N., Lawrence, T.S., Gilula, N.B. and Beers, W.H. (1981) Modulation of cell-to-cell communication in the cumulus–oocyte complex and the regulation of oocyte maturation by LH. *Developmental Biology*, **86**, 356–362.

Dekel, N., Sherizly, I., Tsafriri, A. and Naor, Z. (1983) A comparative study of the mechanism of action of LH and a GnRH analog on the ovary. *Biology of Reproduction*, **28**, 161–166.

Dekel, N., Aberdam, E. and Sherizly, I. (1984a) Spontaneous maturation *in vitro* of cumulus-enclosed rat oocytes is inhibited by forskolin. *Biology of Reproduction*, **31**, 244–250.

Dekel, N., Sherizly, I. and Galiani, D. (1984b) Maturation of rat oocytes follows uncoupling in the cumulus–oocyte complex. *Biology of Reproduction*, Suppl.1,**30**, 144, abstract 277.

Dekel, N., Aberdam, E. and Hanski, E. (1985) Invasive bacterial adenylate cyclase maintains the meiotic arrest in isolated rat oocytes in *Proceedings of the Fifth Ovarian Workshop*, (eds

D.O. Toft and R.J. Ryan), Ovarian Workshops, Champaign, Illinois, pp. 65–69.

Dekel, N., Galiani, D. and Sherizly, I. (1988) Dissociation between the inhibitory and the stimulatory action of cAMP on maturation of rat oocytes. *Molecular and Cellular Endocrinology*, **56**, 115–121.

Dekel, N., Aberdam, E., Goren, S., Feldman, B. and Shalfi, R. (1989) Mechanism of action of GnRH-induced oocyte maturation. *Journal of Reproduction and Fertility*, Suppl. 37, 319–327.

De Leon, V., Johnson, A. and Bachvarova, R. (1983) Half-lives and relative amounts of stored and polysomal ribosomes and poly(A)$^+$RNA in mouse oocytes. *Developmental Biology*, **98**, 400–408.

diZerega, G.S. and Hodgen, G.D. (1980) Cessation of folliculogenesis during the primate luteal phase. *Journal of Clinical Endocrinology and Metabolism*, **51**, 158–160.

diZerega, G.S. and Hodgen, G.D. (1981) Folliculogenesis in the primate ovarian cycle. *Endocrine Reviews*, **2**, 27–49.

diZerega, G.S., Marrs, R.P., Campeau, J.D. and Ray, O. (1983a) Human granulosa cell secretion of protein(s) which suppress follicular response to gonadotropins. *Journal of Clinical Endocrinology and Metabolism*, **56**, 147–155.

diZerega, G.S., Campeau, J.D., Nakamura, R.N., Ujita, E.L., Lobo, R. and Marrs, R.P. (1983b) Activity of a human follicular fluid protein(s) in spontaneous and induced ovarian cycles. *Journal of Clinical Endocrinology and Metabolism*, **57**, 838–846.

Donahue, R.P. (1972) The relation of oocyte maturation to ovulation in mammals (eds A.W. Schuetz and J.D. Biggers), *Oogenesis*, University Park Press, Baltimore, pp. 413–438.

Donahue, R.P. and Stern, S. (1968) Follicular cell support of oocyte maturation: Production of pyruvate *in vitro*. *Journal of Reproduction and Fertility*, **17**, 395–398.

Downs, S.M. and Eppig, J.J. (1984) Cyclic adenosine monophosphate and ovarian follicular fluid act synergistically to inhibit mouse oocyte maturation. *Endocrinology*, **114**, 418–427.

Downs, S.M. and Eppig, J.J. (1985) A follicular fluid component prevents gonadotropin reversal of cyclic adenosine monophosphate-dependent meiotic arrest in murine oocytes. *Gamete Research*, **11**, 83–97.

Downs, S.M. and Eppig, J.J. (1987) Induction of mouse oocyte maturation *in vivo* by perturbants of purine metabolism. *Biology of Reproduction*, **36**, 431–437.

Downs, S.M., Coleman, D.L., Ward-Bailey, P.F. and Eppig, J.J. (1985) Hypoxanthine is the principal inhibitor of murine oocyte maturation in a low molecular weight fraction of porcine follicular fluid. *Proceedings of the National Academy of Sciences of the USA*, **82**, 454–458.

Downs, S.M., Schroeder, A.C. and Eppig, J.J. (1986a) The developmental capacity of mouse oocytes following maintenance of meiotic arrest *in vitro*. *Gamete Research*, **15**, 305–316.

Downs, S.M., Coleman, D.L. and Eppig, J.J. (1986b) Maintenance of murine oocyte meiotic arrest: Uptake and metabolism of hypoxanthine and adenosine by cumulus cell-enclosed and denuded oocytes. *Developmental Biology*, **117**, 174–183.

Downs, S.M., Daniel, S.A.J., Bornslaerger, E.A., Hoppe, P.C. and Eppig, J.J. (1989) Maintenance of meiotic arrest in mouse oocytes by purines: Modulation of cAMP levels and cAMP phosphodiesterase activity. *Gamete Research*, **23**, 323–334.

Dufour, J., Cahill, L.P. and Mauléon, P. (1979) Short- and long-term effects of hypophysectomy and unilateral ovariectomy on ovarian follicular populations in sheep. *Journal of Reproduction and Fertility*, **57**, 301–309.

Dunbar, B.S., Liu, C. and Sammons, D.W. (1981) Identification of the three major proteins of porcine and rabbit zonae pellucidae by high resolution two-dimensional gel electrophoresis: Comparison with serum, follicular fluid, and ovarian cell proteins. *Biology of Reproduction*, **24**, 1111–1124.

Dvòràk, M. and Tesàrik, J. (1980) Ultrastructure of human ovarian follicles in *Biology of the Ovary*, (eds P.M. Motta and E.S.E. Hafez), Nijhoff, The Hague, pp. 121–137.

Eddy, E.M. (1974) Fine structural observations on the form and distribution of nuage in the germ cells of the rat. *Anatomical Record*, **178**, 731–758.

Eddy, E.M. and Clark, J.M. (1975) Electron microscopic study of migrating germ cells in the rat in *Electron Microscopic Concepts of Secretion, Ultrastructure of Endocrine and Reproductive Organs*, (ed. M. Hess), Wiley, New York, pp. 151–167.

Eddy, E.M., Clark, J.M., Gong, D. and Fenderson, B.A. (1981) Origin and migration of primordial germ cells in mammals. *Gamete Research*, **4**, 333–362.

Edwards, R.G. and Gates, A.H. (1959) Timing of

the stages of the maturation, division, ovulation, fertilization and the first cleavage of eggs of adult mice treated with gonadotropins. *Journal of Endocrinology*, **18**, 292–304.

Ekholm, C. and Magnusson, C. (1979) Rat oocyte maturation: effects of protein synthesis inhibitors. *Biology of Reproduction*, **21**, 1287–1293.

Ekholm C, Hillensjö, T. and Isaksson, O. (1981) Gonadotropin and releasing hormone agonists stimulate oocyte meiosis and ovulation in hypophysectomized rats. *Endocrinology*, **108**, 2022–2024.

Ekholm, C., Clark, M.R., Magnusson, C., Isaksson, O. and LeMaire, W.J. (1982) Ovulation induced by a GnRH analog in hypophysectomized rats involves prostaglandins. *Endocrinology*, **110**, 228–290.

Ekholm, C., Hillensjö, T., Magnusson, C. and Rosberg, S. (1984) Stimulation and inhibition of rat oocyte meiosis by forskolin. *Biology of Reproduction*, **30**, 537–543.

Endo, Y., Kopf, G.S. and Schultz, R.M. (1986) Stage-specific changes in protein phosphorylation accompanying meiotic maturation of mouse oocytes and fertilization of mouse eggs. *Journal of Experimental Zoology*, **239**, 401–409.

Eppig, J.J. (1977) Mouse oocyte development *in vitro* with various culture systems. *Developmental Biology*, **60**, 371–388.

Eppig, J.J. (1979) A comparison between oocyte growth in co-culture with granulosa cells and oocytes with granulosa cell–oocyte junctional contact maintained. *Journal of Experimental Zoology*, **209**, 345–353.

Eppig, J.J. (1982) The relationship between cumulus cell–oocyte coupling, oocyte meiotic maturation, and cumulus expansion. *Developmental Biology*, **89**, 268–272.

Eppig, J.J. (1989) The participation of cyclic adenosine monophosphate (cAMP) in the regulation of meiotic maturation of oocytes in the laboratory mouse. *Journal of Reproduction and Fertility*, Suppl. 38, 3–8.

Eppig, J.J. (1991) Mammalian oocyte development *in vivo* and *in vitro*, in *Elements of Mammalian Fertilization* (ed. P.M. Wassarman) CRC Press, Boca Raton, pp. 57–76.

Eppig, J.J. and Downs, S.M. (1984) Chemical signals that regulate mammalian oocyte maturation. *Biology of Reproduction*, **30**, 1–12.

Eppig, J.J. and Downs, S.M. (1987) The effect of hypoxanthine on mouse oocyte growth and development *in vitro*: Maintenance of meiotic arrest and gonadotropin-induced oocyte maturation. *Developmental Biology*, **119**, 313–321.

Eppig, J.J., Freter, R.R., Ward-Bailey, R.F. and Schultz, R.M. (1983) Inhibition of oocyte maturation in the mouse: Participation of cAMP, steroid hormones, and a putative maturation-inhibitory factor. *Developmental Biology*, **100**, 39–49.

Eppig, J.J., Ward-Bailey, P.F. and Coleman, D.L. (1985) Hypoxanthine and adenosine in murine ovarian follicular fluid: Concentrations and activity in maintaining oocyte meiotic arrest. *Biology of Reproduction*, **33**, 1041–1049.

Erickson, B.H. (1966) Development and radioresponse of the prenatal bovine ovary. *Journal of Reproduction and Fertility*, **11**, 97–105.

Erickson, G.F. and Ryan, K.J. (1975) The effect of LH/FSH, dibutyryl cyclic AMP, and prostaglandins on the production of estrogens by rabbit granulosa cells *in vitro*. *Endocrinology*, **97**, 108–113.

Erickson, G.F. and Ryan, K.J. (1976a) Stimulation of testosterone production in isolated rabbit thecal tissue by LH/FSH, dibutyryl cyclic AMP, $PGF_{2\alpha}$ and PGF_2. *Endocrinology*, **99**, 452–458.

Erickson, G.F. and Ryan, K.J. (1976b) Spontaneous maturation of oocytes isolated from ovaries of immature hypophysectomized rats. *Journal of Experimental Zoology*, **195**, 153–158.

Erickson, G.F. and Sorenson, R.A. (1974) *In vitro* maturation of mouse oocytes isolated from ovaries of immature hypophysectomized rats. *Journal of Experimental Zoology*, **190**, 123–127.

Erickson, G.F., Magoffin, D.A., Dyer, C.A. and Hofeditz, D. (1985) The ovarian androgen producing cells: A Review of structure/function relationships. *Endocrine Reviews*, **6**, 371–399.

Esch, F.S., Shimasaki, S., Mercado M., Cooksey, K., Ling, N., Ying, S., Ueno, N. and Guillemin, R. (1987) Structural characterization of follistatin: A novel follicle-stimulating hormone release-inhibiting polypeptide from the gonad. *Molecular Endocrinology*, **1**, 849–855.

Eshkol, A. and Lunenfeld, B. (1972) Gonadotrophic regulation of ovarian development in mice during infancy in *Saxena BB*, (eds C.G. Berling and H.M. Gandy), *Gonadotropins*, Wiley Interscience, New York, pp. 335–346.

Eshkol, A., Lunenfeld, B. and Peters, H. (1970) Ovarian development in infant mice. Dependence on gonadotropic hormones, in *Gonadotropins and Ovarian Development*, (eds

W.R. Butt and A.C. Crooke), E & S Livingstone, Edinburgh, pp. 249–258.

Espey, L.L. (1967a) Ultrastructure of the apex of the rabbit Graafian follicle during the ovulatory process. *Endocrinology*, **81**, 267–277.

Espey, L.L. (1967b) Tenacity of porcine Graafian follicle as it approaches ovulation. *American Journal of Physiology*, **212**, 1397–1401.

Espey, L.L. (1971a) Decomposition of connective tissue in rabbit ovarian follicles by multivesticular structures of thecal fibroblasts. *Endocrinology*, **88**, 437–444.

Espey, L.L. (1971b) Multivesticular structures in proliferating fibroblasts of rabbit ovarian follicles during ovulation. *Journal of Cell Biology*, **48**, 437–442.

Espey, L.L. (1974) Ovarian proteolytic enzymes and ovulation. *Biology of Reproduction*, **10**, 216–235.

Espey, L.L. (1975) Evaluation of proteolytic activity in mammalian ovulation in *Protease and Biological Control*, (eds E. Reich, D.B. Rifkin and E. Shaw), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 767–776.

Espey, L.L. (1978) Ovulation in *The Vertebrate Ovary* (ed. R.E. Jones), Plenum Press, New York, pp. 503–532.

Espey, L.L. (1980) Ovulation as an inflammatory reaction – a hypothesis. *Biology of Reproduction*, **22**, 73–106.

Espey, L.L. and Coons, P.J. (1976) Factors which influence ovulatory degradation of rabbit ovarian follicles. *Biology of Reproduction*, **14**, 233–245.

Espey, L.L. and Lipner, H. (1963) Measurements of intrafollicular pressures in the rabbit ovary. *American Journal of Physiology*, **205**, 1067–1072.

Espey, L.L. and Lipner, H. (1965) Enzyme-induced rupture of rabbit Graafian follicle. *American Journal of Physiology*, **208**, 208–213.

Espey, L.L. and Rondell, P. (1967) Estimation of mammalian collagenolytic activity with a synthetic substrate. *Journal of Applied Physiology*, **23**, 457–461.

Espey, L. and Stacy, S. (1970) Failure of an ovarian collagenolytic extract to decompose the connective tissue in the mature sow Graafian follicle. *Federation Proceedings*, **29**, 833.

Espey, L.L., Coons, P.J., Marsh, J.M. and LeMaire, W.J. (1981) Effect of indomethacin on preovulatory changes in the ultrastructure of rabbit Graafian follicles. *Endocrinology*, **108**, 1040–1048.

Espey, L. Shimada, H., Okamura, H. and Mori, T. (1985) Effect of various agents on ovarian plasminogen activator activity during ovulation in PMSG-primed immature rats. *Biology of Reproduction*, **32**, 1087–1094.

Espey, L.L., Tanaka, N., Woodard, D.S., Harper, M.J.K. and Okamura, H. (1989) Decrease in ovarian platelet-activating factor during ovulation in the gonadotropin-primed immature rat. *Biology of Reproduction*, **40**, 104–110.

Evans, C.W., Robb, D.I., Tuckett, F. and Challoner, S. (1982) Regulation of meiosis in the fetal mouse gonad. *Journal of Embryology and Experimental Morphology*, **68**, 59–67.

Everett, N.B. (1943) Observational and experimental evidences relating to the origin and differentiation of the definitive germ cells in mice. *Journal of Experimental Zoology*, **92**, 49–91.

Fajer, A.B., Schneider, J., McCall, D., Anges, I.G. and Plakis, S.E. (1979) The induction of meiosis by ovaries of newborn hamsters and its relation to the action of the extra ovarian structures in the meosvarium (rete ovarii). *Annales de Biologie animale, Biochimie et Biophysique*, **19**, 1273–1278.

Falck, B. (1959) Site of production of oestrogen in rat ovary as studied in micro-transplants. *Acta Physiologica Scandinavica*, **47**, Suppl 163, 1–100.

Feldman, E., Haberman, S., Abisogun, A.O., Reich, R., Levran, D., Maschiach, S., Zuckerman, H., Rudak, E., Dor, J. and Tsafriri, A. (1986) Arachidonic acid metabolism in human granulosa cells: Evidence for cyclooxygenase and lipoxygenase activity *in vitro*. *Human Reproduction*, **1**, 353–356.

Findlay, J.K. and Risbridger, G.P. (1987) Intra-gonadal control mechanisms in *Bailliere's Clinical Endocrinology and Metabolism: Reproductive Endocrinology*, (ed. H.G. Burger), Bailliere Tindall, London, pp. 223–243.

Finidori-Lepicard, J., Schorderet-Slatkine, S., Hanoune, J. and Baulieu, E.E. (1981) Progesterone inhibits membrane-bound adenylate cyclase in *Xenopus laevis* oocytes. *Nature* **292**, 255–257.

Fleming, A.D., Kuehl, T.J. and Armstrong, D.T. (1985) Maturation of pig and rat oocytes transplanted into surrogate pig follicles *in vitro*. *Gamete Research*, **11**, 107–119.

Fletcher, W.H. (1979) Intercellular junctions in ovarian follicles: A possible functional role in follicle development in *Ovarian Follicular Development and Function*, (eds A.R. Midgley and W.A. Sadler), Raven Press, New York, pp. 113–120.

Foote, W.D. and Thibault, C. (1969) Recherches expérimentales sur la maturation *in vitro* des ovocytes de truie et de veau. *Annales de Biologie Animale, Biochimie et Biophysique*, **9**, 329–349.

Fortune, J.E. (1986) Bovine theca cells interact to promote androgen production. *Biology of Reproduction*, **35**, 292–299.

Fortune, J.E. and Armstrong, D.T. (1977) Androgen production by theca granulosa isolated from pro-oestrous rat follicles. *Endocrinology*, **100**, 1241–1247.

Francavilla, S. and Zamboni, L. (1985) Differentiation of mouse ectopic germinal cells in intra- and perigonadal locations. *Journal of Experimental Zoology*, **233**, 101–109.

Franchi, L.L. and Mandl, A.M. (1962) The ultrastructure of oogonia and oocytes in the foetal and neonatal rat. *Proceedings of the Royal Society of London Series B*, **157**, 99–114.

Franchi, L.L., Mandl, A.M. and Zuckerman, S. (1962) The development of the ovary and the process of oogenesis in *The Ovary*, 1st edn, Vol. 1, (ed. S. Zuckerman), Academic Press, New York, pp. 1–88.

Freter, R.R. and Schultz, R.M. (1984) Regulation of murine oocyte meiosis: Evidence for a gonadotropin-induced, cAMP-dependent reduction in a maturation inhibitor. *Journal of Cell Biology*, **98**, 1119–1128.

Fujimoto, T., Miyayama, Y. and Fuyuta, M. (1977) The origin, migration and fine morphology of human primordial germ cells. *Anatomical Record*, **188**, 315–330.

Fukumoto, M., Yajima, Y., Okamura, H. and Midorikawa, O. (1981) Collagenolytic enzyme activity in human ovary: An ovulatory enzyme system. *Fertility and Sterility*, **36**, 746–750.

Fulka Jr., J. (1985) Maturation-inhibiting activity in growing mouse oocytes. *Cell Differentiation*, **17**, 45–48.

Fulka Jr., J., Motlik, J. and Crozet, N. (1985) Inhibition of nuclear maturation in fully grown porcine and mouse oocytes after fusion with growing porcine oocytes. *Journal of Experimental Zoology*, **235**, 255–259.

Fulka Jr., J., Motlik, J., Fulka, J. and Jilek, F. (1986) Effect of cycloheximide on nuclear maturation of pig and mouse oocytes. *Journal of Reproduction and Fertility*, **77**, 281–285.

Funkenstein, B. and Nimrod, A. (1981) Control of developmental processes in cultured neonatal ovaries in *Development and Function of Reproductive Organs*, International Congress Series No. 559 (eds A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 307–318.

Funkenstein, B., Nimrod A. and Lindner, H.R. (1980) The development of steroidogenic capability and responsiveness to gonadotropins in cultured neonatal rat ovaries. *Endocrinology*, **106**, 98–106.

Galway, A.B., LaPolt, P.S., Tsafriri, A., Dargan, C.M., Boime, I. and Hsueh, A.J.W. (1990) Recombinant FSH induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. *Endocrinology*, **127**, 3023–3028.

Gardner, R.L., Lyon, M.F., Evans, E.P. and Burtenshaw, M.D. (1985) Clonal analysis of X-chromosome inactivation and the origin of the germ line in mouse embryo. *Journal of Embryology and Experimental Morphology*, **88**, 349–363.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J.L. (1988) Purified maturation promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene cdc2+. *Cell*, **54**, 433–439.

Gautier, J., Matsukawa, T., Nurse, P. and Maller, J.L. (1989) Dephosphorylation and activation of *Xenopus* $p34_{cdc2}$ protein kinase during the cell cycle. *Nature*, **339**, 626–629.

Gelehrter, T.D., Sznycer-Lascuk, R., Zeheb, R. and Cwikel, B.J. (1987) Dexamethasone inhibition of tissue-type plasminogen activator (tPA) activity: Paradoxical induction of both tPA antigen and plasminogen activator inhibitor. *Molecular Endocrinology*, **1**, 97–101.

Gérard, M., Menezo, Y., Rombauts, P., Szöllösi, D. and Thibault, C. (1979) *In vitro* studies of oocyte maturation and follicular metabolism in the pig. *Annales de Biologie Animale, Biochimie et Biophysique*, 1521–1535.

Gerhart, J. Wu, M. and Kirshner, M. (1984) Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. *Journal of Cell Biology*, **98**, 1247–1255.

Gillim, S.W., Christensen, A.K. and McLennan, C.E. (1969) Fine structure of the human menstrual corpus luteum at its stage of maximum secretory activity. *American Journal of Anatomy*, **126**, 409–428.

Gilula, N.B., Epstein, M.L. and Beers, W.H. (1978) Cell-to-cell communication and ovulation: A study of the cumulus–oocyte complex. *Journal of Cell Biology*, **78**, 58–75.

Ginsburg, M., Snow, M.H.L. and McLaren, A.

(1990) Primordial germ cells in the mouse embryo during gastrulation. *Development*, **110**, 521–528.

Glass, L.E. (1971) Transmission of maternal proteins into oocytes. *Advances in Bioscience*, **6**, 29–58.

Goetz, F.W., Berndtson, A.K. and Ranjan, M. (1991) Ovulation: mediators at the ovarian level, in *Vertebrate Endocrinology: Fundamental and Biomedical Implications* (eds P.K.T. Pand, M.P. Schreibman and R. Jones) Academic Press, New York, pp. 127–203.

Golbus, M.S. and Stein, M.P. (1976) Qualitative patterns of protein synthesis in the mouse oocytes. *Journal of Experimental Zoology*, **198**, 337–342.

Goldenberg, R.L., Vaitukaitus, J.L. and Ross, G.T. (1972) Estrogen and follicle stimulating hormone interactions on follicle growth in rats. *Endocrinology*, **90**, 1492–1498.

Gondos, B. (1969) Ultrastructure of the germinal epithelium during oogenesis in the rabbit. *Journal of Experimental Zoology*, **172**, 465–480.

Gondos, B. (1973) Germ cell degeneration and intercellular bridges in the human fetal ovary. *Zeitschrift fur Zellforschung Mikroskopie Anatomie*, **138**, 23–30.

Gondos, B. (1975) Surface epithelium of the developing ovary. *American Journal of Pathology*, **81**, 303–312.

Gondos, B. (1978) Oogonia and oocytes in mammals in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 83–120.

Gondos, B. and Zamboni, L. (1969) Ovarian development: the functional importance of germ cell interconnections. *Fertility and Sterility*, **20**, 176–189.

Gondos, B., Bhiraleus, P. and Hobel, C.J. (1971) Ultrastructural observations on germ cells in human fetal ovaries. *American Journal of Obstetrics and Gynecology*, **110**, 644–652.

Gondos, B., Westergaard, L. and Byskov, A.G. (1986) Initiation of oogenesis in the human fetal ovary: Ultrastructural and squash preparation study. *American Journal of Obstetrics and Gynecology*, **155**, 189–195.

Goodman, A.L. and Hodgen, G.D. (1983) The ovarian triad of the primate menstrual cycle. *Recent Progress in Hormone Research*, **34**, 1–73.

Goodman, A.L., Nixon, W.E. and Hodgen, G.D. (1979) Between-ovary interaction in the regulation of follicle growth, corpus luteum function, and gonadotropin secretion in the primate ovarian cycle. III. Temporal and spatial dissociation of folliculogenesis and negative feedback regulation of tonic gonadotropin release after luteectomy in rhesus monkeys. *Endocrinology*, **105**, 69–73.

Goodman, A.L., Nixon, W.E., Johnson, D.K. and Hodgen, G.D. (1977) Regulation of folliculogenesis in the cycling rhesus monkey: selection of the dominant follicle. *Endocrinology*, **100**, 155–161.

Gordon, I. and Lu, K.H. (1990) Production of embryos *in vitro* and its impact on livestock production. *Theriogenology*, **33**, 77–87.

Gore-Langton, R.E. and Armstrong, D.T. (1988) Follicular steroidogenesis and its control, in *Physiology of Reproduction* (eds E. Knobil and J. Neill) Raven Press, New York, pp. 331–385.

Gougeon, A. (1982) Rate of follicular growth in the human ovary in *Follicular Maturation and Ovulation* (eds R. Rolland, E.V. Van Hall, S.G. Hillier, K.P. McNatty and J.S. Schoemaker), Excerpta Medica, Amsterdam, pp. 155–163.

Gougeon, A. (1986) Dynamics of follicular growth in the human: a model from preliminary results. *Human Reproduction*, **1**, 81–87.

Greenwald, G.S. and Terranova, P.F. (1988) Follicular selection and its control, in *Physiology of Reproduction* (eds E. Knobil and J. Neill) Raven Press, New York, pp. 387–445.

Greve, J.M., Salzman, G.S., Roller, R.J. and Wassarman, P.M. (1982) Biosynthesis of the major zona pellucida glycoprotein secreted by oocytes during mammalian oogenesis. *Cell*, **31**, 749–759.

Grinsted, J., Byskov, A.G. and Andreasen, M.P. (1979) Induction of meiosis in fetal mouse testis *in vitro*, by rete testis tissue from pubertal mice and bulls. *Journal of Reproduction and Fertility*, **56**, 653–656.

Grinwich, D.L., Kennedy, T.G. and Armstrong, D.T. (1972) Dissociation of ovulatory and steroidogenic actions of luteinizing hormone in rabbits with indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins*, **1**, 89–96.

Grumbach, M.M. and Kaplan, S.L. (1973) Ontogenesis of growth hormone, insulin, prolactin and gonadotrophin secretion in the human fetus in *Fetal and Neonatal Physiology*, Cambridge, Cambridge University Press, pp. 362–387.

Gulyas, B.J., Hodgen, G.D., Tullner, W.W. and Ross, G.T. (1977) Effects of fetal or maternal hypophysectomy on endocrine organs and body

weight in infant rhesus monkeys (*Macaca mulatta*): with particular emphasis on oogenesis. *Biology of Reproduction*, **16**, 216–227.

Gwatkin, R.B.L. and Andersen, O.F. (1976) Hamster oocyte maturation *in vitro* inhibition by follicular components. *Life Sciences*, **19**, 527–536.

Hage, A.J., Groen-Kelvant, A.C. and Welschen, R. (1978) Follicle growth in the immature rat ovary. *Acta Endocrinologica*, **88**, 375–382.

Hamada, Y., Wright, K.H. and Wallach, E.E. (1979) The effects of progesterone and human chorionic gonadotrophin on ovulation in the *in vitro* perfused rabbit ovary. *Fertility and Sterility*, **32**, 335–339.

Haney, H.F. and Schomberg, D.W. (1981) Estrogen and progesterone production by developing porcine follicles *in vitro*: evidence for estrogen formation by theca. *Endocrinology*, **109**, 971–977.

Hardisty, M.W. (1978) Primordial germ cells in the vertebrate germ line in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 1–45.

Harrington, F.E., Bex, F.J., Elton, R.L. and Roach, J.B. (1970) The ovulatory effects of follicle stimulating hormone treated with chymotrypsin in chlorpromazine blocked rats. *Acta Endocrinologica*, **65**, 222–228.

Harrison, R.J. and Weir, B.J. (1977) Structure of the mammalian ovary in *The Ovary*, 2nd edn (eds S. Zuckerman and B.J. Weir), Academic Press, New York, pp. 113–217.

Hashimoto, H. and Kishimito, T. (1988) Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. *Developmental Biology*, **126**, 242–252.

Hashimoto, N., Kishimoto, T. and Nagahama, Y. (1985) Induction and inhibition of meiotic maturation in follicle-enclosed mouse oocytes by forskolin. *Development, Growth and Differentiation*, **27**, 709–716.

Hashimoto, Y. and Eguchi, Y. (1955a) Histological observations on the gonads in the cattle and the horse fetus. I. The cattle fetus. *Japanese Journal of Zootechnological Science*, **26**, 259–266.

Hashimoto, Y. and Eguchi, Y. (1955b) Histological observations on the gonads in the cattle and the horse fetus. II. The horse fetus. *Japanese Journal of Zootechnological Science*, **26**, 267–272.

Hastings, R.A., Enders, A.C. and Schlafke, S. (1972) Permeability of the zona pellucida to protein tracers. *Biology of Reproduction*, **7**, 288–296.

Hay, M.F. and Moor, R.M. (1973) The Graafian follicle of the sheep: relationship between gonadotropins, steroid production morphology and oocyte maturation. *Annales de Biologie Animale, Biochimie et Biophysique*, **13**, 241–249.

Hayaishi, O. and Yamamoto, S. (eds) (1985) *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 15, Kyoto Conference on Prostaglandins, Raven Press, New York.

Healy, D.L. Bacher, J. and Hodgen, G.D. (1985) Thymic regulation of primate ovarian–adrenal differentiation. *Biology of Reproduction*, **32**, 1127–1133.

Heath, J.K. (1978a) Characterization of a xenogenic antiserum raised against the fetal germ cells of the mouse; cross reactivity with embryonal carcinoma cells. *Cell*, **15**, 299–306.

Heath, J.K. (1978b) Mammalian primordial germ cells. *Development of Mammals*, **3**, 267–298.

Heaton, J.H. and Gelehter, T.D. (1990) Cyclic nucleotide regulation of plasminogen activator-inhibitor messenger RNAs in rat hepatoma cells. *Molecular Endocrinology*, **4**, 171–178.

Hedrick, J.L., Fry, G.N. (1980) Immunocytochemical studies on the porcine zona pellucida. *Journal of Cell Biology*, **87**, 143a.

Hekman, C.M. and Loskutoff, D.J. (1988) Bovine plasminogen activator inhibitor 1: Specificity determinations and comparison of the active, latent and guanidine-activated forms. *Biochemistry*, **27**, 2911–2918.

Heller, D.T. and Schultz, R.M. (1980) Ribonucleoside metabolism by mouse oocytes: Metabolic cooperativity between the fully grown oocyte and cumulus cells. *Journal of Experimental Zoology*, **214**, 355–364.

Heller, D.T., Cahill, D.M. and Schultz, R.M. (1981) Biochemical studies of mammalian oogenesis: Metabolic cooperativity between granulosa cells and growing mouse oocytes. *Developmental Biology*, **84**, 455–464.

Henderson, S.A. and Edwards, R.G. (1968) Chiasma frequency and maternal age in mammals. *Nature*, **218**, 22–28.

Henricson, B. and Rajakoski, E. (1959) Studies of oocytogenesis in cattle. *Cornell Veterinary*, **49**, 494–503.

Hensleigh, H.C. and Hunter, A.G. (1985) *In vitro* maturation of bovine cumulus enclosed primary oocytes and their subsequent *in vitro* fertilization and cleavage. *Journal of Dairy Science*, **68**, 1456–1462.

Herlands, R.L. and Schultz, R.M. (1984) Regulation of mouse oocyte growth: Probable

nutritional role for intercellular communication between follicle cells and oocytes in oocyte growth. *Journal of Experimental Zoology*, **229**, 317–325.

Herlant, M. (1961) L'activité génitale chez la de *Galago senegalensis moholi* (Geoffr.) et ses rapports avec la persistance de phénomebes d'ovogenese chez l'adulte. *Annals of the Royal Zoological Society of Belgium*, **91**, 1–15.

Hertig, A.T. and Adams, E.C. (1967) Studies on the human oocyte and its follicle. Ultrastructural and cytochemical observations on the primordial follicle stage. *Journal of Cell Biology*, **34**, 647–675.

Hertzberg, E.G., Lawrence, T.S. and Gilula, N.B. (1981) Gap junctional communication. *Annual Review of Physiology*, **43**, 479–491.

Hild-Petito, S., Stouffer, R.L. and Brenner, R.M. (1988) Immunocytochemical localization of estradiol and progesterone receptors in the monkey ovary throughout the menstrual cycle. *Endocrinology*, **123**, 2896–2905.

Hillensjö, T. (1976) Oocyte maturation and glycolysis in isolated pre-ovulatory follicles of PMS-injected immature rats. *Acta Endocrinologica*, **82**, 809–830.

Hillensjö, T. (1981) Steroid biosynthesis by granulosa, thecal and stromal cells: Their interaction in *Intragonadal Regulation of Reproduction*, (eds P. Franchimont and C.P. Channing), Academic Press, London, pp. 33–60.

Hillensjö, T. and Channing, C.P. (1980) Gonadotropin stimulation of steroidogenesis and cellular dispersion in cultured porcine cumuli oophori. *Gamete Research*, **3**, 233–240.

Hillensjö, T. and LeMaire, W.J. (1980) Gonadotropin-releasing hormone agonists stimulate meiotic maturation of follicle-enclosed rat oocytes *in vitro*. *Nature*, **287**, 145–156.

Hillensjö, T., Batta, S.K., Schwartz-Kripner, A., Wentz, A.C., Sulewski, J. and Channing, C.P. (1978a) Inhibitory effect of human follicular fluid upon the maturation of porcine oocytes in culture. *Journal of Clinical Endocrinology and Metabolism*, **47**, 1332–1335.

Hillensjö T., Ekholm, C. and Ahrén, K. (1978b) Role of cyclic AMP in oocyte maturation and glycolysis in the pre-ovulatory rat follicle. *Acta Endocrinologica*, **87**, 377–388.

Hillensjö, T., Channing, C.P., Pomerantz, S.H. and Schwartz-Kripner, A. (1979a) Intrafollicular control of oocyte maturation in the pig. *In Vitro*, **15**, 32–40.

Hillensjö, T., Kripner, A.S., Pomerantz, S.H. and Channing, C.P. (1979b) Action of porcine follicular fluid oocyte maturation *in vitro*: possible role of the cumulus cells in *Ovarian Follicular and Corpus Luteum Function* (eds C.P. Channing, J.M. Marsh and W.A. Sadler), Plenum Press, New York, pp. 283–291.

Hillensjö, T., Pomerantz, S.H., Schwartz-Kripner, A., Anderson, L.D. and Channing, P.D. (1980) Inhibition of cumulus cell progesterone secretion by low molecular weight fractions of porcine follicular fluid which also inhibit oocyte maturation. *Endocrinology*, **106**, 584–591.

Hillensjö, T., Brännström, M., Chari, S., Daume, E., Magnusson, C., Nilsson, L., Sjögren, A. and Törnell, J. (1985) Oocyte maturation as regulated by follicular factors. *Annals of the New York Academy of Science*, **442**, 73–79.

Hilscher, B. and Hilscher, W. (1978) Kinetics of gametogenesis. II. Comparative autoradiographic studies of oogonia and multiplying prospermatogonia of the Wistar rat. *Cell and Tissue Research*, **190**, 61–68.

Hilscher, B. and Hilscher, W. (1981) Some details on the kinetics of oogonia and M-prospermatogonia in NMRI mice in comparison to rat and man in *Development and Function of Reproductive Organs*, (eds A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 131–139.

Hilscher, B., Hilscher, W., Bulthoff, Ohnolz, B., Kramer, U., Birke, A., Pelzer, H. and Gauss, G. (1974) Kinetics of gametogenesis. I. Comparative histological and autoradiographic studies of oocytes and transitional propsermatogonia during oogenesis and prespermategonesis. *Cell Tissue Research*, **154**, 443–470.

Himelstein-Braw, R., Byskov, A.G., Peters, H. and Faber, M. (1976) Follicular atresia in the infant human ovary. *Journal of Reproduction and Fertility*, **46**, 55–59.

Hirshfield, A.N. (1986) Patterns of [^{3}H] thymidine incorporation differ in immature rats and mature, cycling rats. *Biology of Reproduction*, **34**, 229–235.

Hirshfield, A.N. (1987) In: *Development and Function of Reproductive Organs*, Vol. III, (eds M. Parvinen, I. Huhtaniemi and L.J. Pelliniemi) Ares-Serono Symposia, Rome, pp. 171–182.

Hirshfield, A.N. (1991) Development of follicles in the mammalian ovary. In *International Review of Cytology. A Survey of Cell Biology* (eds K.W. Jeon and M. Friedlander) Academic Press, Inc., San Diego, California, pp. 43–101.

Hirshfield, A.N. and Midgley Jr., A.R. (1978a) Morphometric analysis of follicular development in the rat. *Biology of Reproduction*, **19**, 597–605.

Hirshfield, A.N. and Midgley Jr., A.R. (1978b) The role of FSH in the selection of large ovarian follicles in the rat. *Biology of Reproduction*, **19**, 606–611.

Hirshfield, A.N. and Schmidt, W.A. (1987) Kinetic aspects of follicular development in the rat in *Regulation of Ovarian and Testicular Function*, (ed. V.B. Mahesh), Plenum Press, New York, pp. 211–236.

Hodgen, G.D. (1982) The dominant ovarian follicle. *Fertility and Sterility*, **38**, 281–300.

Holm, P.B. and Rasmussen, S.W. (1977) Human meiosis. I. The human pachytene karyotype analyzed by three dimensional reconstruction of the synaptonemal complex. *Carlsberg Research Communication*, **42**, 283–323.

Holmes, P.V., Janson, P.O., Sogn, J., Kallfelt, B., LeMaire, W.J., Ahren, K., Cajander, S. and Bjersing, L. (1983) Effects of PGF_2 and indomethacin on ovulation and steroid production in the isolated perfused rabbit ovary. *Acta Endocrinologica*, **104**, 233–239.

Holmes, P.V., Sogn, J., Schillinger, E. and Janson, P.O. (1985) Effects of high and low preovulatory concentrations of progesterone on ovulation from the isolated perfused rabbit ovary. *Journal of Reproduction and Fertility*, **75**, 393–399.

Hope, J. (1965) The fine structure of the developing follicle of the rhesus ovary. *Journal of Ultrastructural Research*, **12**, 592–610.

Hubbard, C.J. (1985) The effects of forskolin and LH on cAMP changes and maturation in the follicle-enclosed oocytes of hamsters. *Acta Endocrinologica*, **110**, 413–420.

Hubbard, C.J. and Terranova, P.F. (1982) Inhibitory action of cyclic guanosine 5-phosphoric acid (GMP) on oocyte maturation. Dependent on an intact cumulus. *Biology of Reproduction*, **26**, 628–632.

Hunter, R.H.F. and Polge, C. (1966) Maturation of follicular oocytes in the pig after injection of human chorionic gonadotropin. *Journal of Reproduction and Fertility*, **12**, 525–531.

Hunter, R.H.F., Cook, B. and Baker, T.G. (1976) Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs. *Nature*, **260**, 156–158.

Ichikawa, S., Morioka, H., Ohta, M., Oda, K. and Murao, S. (1983a) Effect of various proteinase inhibitors on ovulation of explanted hamster ovaries. *Journal of Reproduction and Fertility*, **68**, 17–19.

Ichikawa, S., Ohta, M., Morioka, H. and Murao, S. (1983b) Blockage of ovulation in the explanted hamster ovary by a collagenase inhibitor. *Journal of Reproduction and Fertility*, **68**, 17–19.

Inano, H., Inano, A and Tamaoki, B. (1970) Studies on enzyme reactions related to steroid biosynthesis. II. Submicrosomal distribution of the enzymes related to androgen production from pregnenolone and of the cytochrome P-450 in testicular gland of rat. *Journal of Steroid Biochemistry*, **1**, 83–91.

Ingram, D.L. (1962) Atresia in *The Ovary*, 1st edn, Vol. 1, (ed. S. Zuckerman), Academic Press, New York, pp. 247–273.

Ioannou, J.M. (1964) Oogenesis in the guinea pig. *Journal of Embryology and Experimental Morphology*, **12**, 673–691.

Ioannou, J.M. (1967) Oogenesis in adult prosimias. *Journal of Embryology and Experimental Morphology*, **17**, 139–145.

Ireland, J.J. and Richards, J.S. (1978) A previously undescribed role for luteinizing hormone (LH:hCG) on follicular cell differentiation. *Endocrinology*, **102**, 1458–1465.

Iwamasa, J., Shibata, S., Tanaka, N., Matsuura, K. and Okamure, H. (1992) The relationship between ovarian progesterone and proteolytic enzyme activity during ovulation in the gonadotrophin-treated rat. *Biology of Reproduction*, **46**, 309–313.

Iwamatsu, T. and Yanagimachi, R. (1975) Maturation *in vitro* of ovarian oocytes of prepubertal and adult hamsters. *Journal of Reproduction and Fertility*, **45**, 83–90.

Iyengar, M.R., Iyengar, C.W.L., Chen, H.Y., Brinster, R.L., Bornslaeger, E. and Schultz, R.M. (1983) Expression of creatine kinase isoenzyme during oogenesis and embryogenesis in the mouse. *Developmental Biology*, **96**, 263–268.

Jacobs, H.S., Abdulwahid, N., Adams, J., Armar, N.A., Brook, C.G.D., Chambers, G.R., Craft, I., Franks, S., Jeffcoat, S.L., Mason, A., Mason, W.P., Morris, D.V., Porter, R., Stanhope, R., Sutherland, I. and Van Derspay, Z.N. (1985) Endocrine control of follicular growth in humans in *Gamete Quality and Fertility Regulation*, (eds R. Rolland, M.J. Heinemann, S.G. Hillier and H. Vemer), Excerpta Medica, Amsterdam, pp. 257–270.

Jagiello, G.M. (1969) Inhibition of reduction in

female mouse meiosis. *Experientia*, **25**, 695–696.

Jagiello, G., Graffeo, J., Ducayen, M. and Prosser, R. (1977) Further studies of inhibitors of in vitro mammalian oocyte maturation. *Fertility and Sterility*, **28**, 476–481.

Jahn, C.L., Baran, M.M. and Bachvarova, R. (1976) Stability of RNA synthesized by the mouse oocyte during its major growth phase. *Journal of Experimental Zoology*, **197**, 161–172.

Janson, P.O. (1975) Effects of luteinizing hormone on blood in the follicular rabbit ovary as measured by radioactive microspheres. *Acta Endocrinologica*, **79**, 122–123.

Janson, P.O., LeMaire, W.J., Källfelt, B., Holmes, P.V., Cajander, S., Bjersing, L., Wiqvist, N. and Ahrén, K (1982) The study of ovulation in the isolated perfused rabbit ovary. I. Methodology and patterns of steroidogenesis. *Biology of Reproduction*, **26**, 456–465.

Jeon, U.W. and Kennedy, J.R. (1973) The primordial germ cells in early mouse embryos; light and electron microscopic studies. *Developmental Biology*, **31**, 275–284.

Jeppesen, T. (1975) Surface epithelium of the fetal guinea pig ovary. A light and electron microscopic study. *Anatomical Record*, **183**, 499–515.

Johnson, M.H., Eager, D., Muggleton-Harris, A.L. and Graves, H.M. (1975) Mosaicism in the organization of concanavalin A receptors on surface membrane of mouse eggs. *Nature*, **257**, 321–322.

Jones, R.E. (1978) Evolution of the vertebrate ovary: An overview in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 763–788.

Jordana, X., Allende, C.C. and Allende, J.E. (1981) Guanine nucleotides are required for progesterone inhibition of amphibian oocyte adenylate cyclase. *Biochemistry International*, **3**, 527–532.

Jost, A. and Prepin, J. (1966) Donnes sur la migration des cellules germinales primordiales du foetus de veau. *Archives d'Anatomie Microscopique et de Morphologie Experimentale*, **55**, 161–186.

Kahnt, F.W. and Neher, R. (1966) Uber die adrenale steroid-biosynthese *in vitro*. III. Selektive Hemmung der nebennierenrinden-funktion. *Helvetica Chimica Acta*, **49**, 725–732.

Kaji, E., Bornslaeger, E.A. and Schultz, R.M. (1987) Inhibition of mouse oocyte cyclic nucleotide phosphodiesterase by steroid hormones: a possible mechanism for steroid hormone inhibition of oocyte maturation. *Journal of Experimental Zoology*, **243**, 489–493.

Kammerman, S. and Ross, J. (1975) Increase in numbers of gonadotropin receptors on granulosa cells during follicle maturation. *Journal of Clinical Endocrinology and Metabolism*, **41**, 546–550.

Kang, Y.H. (1974) Development of the zona pellucida in the rat oocyte. *American Journal of Anatomy*, **139**, 535–566.

Kaplan, G., Abreu, S.L. and Bachvarova, R. (1982) rRNA accumulation and protein synthetic patterns in growing mouse oocytes. *Journal of Experimental Zoology*, **220**, 361–370.

Kawamura, N., Himeno, N., Okamura, H. and Mori, T. (1986) Effect of $PGF_{2\alpha}$ on ovulation and collagenolytic activities in the rabbit treated by indomethacin. *Acta Obstetrica Gynaecologica Japonica*, **38**, 81–87.

Kelly, S.J., (1977) Studies of the developmental potential of 4- and 8-cell stage mouse blastomeres. *Journal of Experimental Zoology*, **200**, 365–375.

Kennelly, J.J. and Foote, R.H. (1966) Oocytogenesis in rabbits: the role of neogenesis in the formation of definite ova and the stability of oocyte DNA measured with tritiated thymidine. *American Journal of Anatomy*, **118**, 573–585.

Kennelly, J.J., Foote, R.H. and Jones, R.C. (1970) Duration of premeiotic deoxyribonucleic acid synthesis and the stages of prophase in rabbit oocytes. *Journal of Cell Biology*, **47**, 577–584.

Kerin, J.F., Edmonds, D.K., Warnes, G.M., Cox, L.W., Seamark, R.F., Mathews, C.G., Young, G.B. and Baird, D.T. (1981) Morphological and functional relations of Graafian follicle growth to ovulation in women using ultrasonic, laparascopic and biochemical measurements. *British Journal of Obstetrics and Gynaecology*, **88**, 81–90.

Kikukawa, Y. Ishikawa, M., Sengoku, K., Kawamo, M. and Shimizu, T. (1991) The effect of platelet activating factor on ovulation. *Prostaglandins*, **42**, 95–104.

Killick, S. and Elstein, M. (1987) Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. *Fertility and Sterility*, **47**, 773–777.

Kishimoto, T., Yamazaki, K., Kato, Y., Koide, S.S. and Kanatani, H. (1984) Induction of starfish maturation by maturation promoting factor of mouse and surf clam oocytes. *Journal of Experimental Zoology*, **231**, 293–295.

Koering, M.J. (1969) Cyclic changes in ovarian morphology during the menstrual cycle in Macaca mulatta. *American Journal of Anatomy*, **126**, 73–101.

Kolena, J. (1976) Ontogenic development of the responsiveness in cAMP synthesis to LH and PGE_2 and gonadotropin receptors in the rat ovary. *Biology of the Neonate*, **29**, 96–103.

von Kölliker, A. (1898) Uber die Markkanale und Markstrange in den Eierstocken junger Hundinnen. *Verhandlungen des Anatomische Gesellshaft*, **14**, 149–156.

Koos, R.D., Feiertag, M.A., Brodie, A.M.H. and LeMaire, W.J. (1984) Inhibition of estrogen synthesis does not inhibit luteinizing hormone-induced ovulation. *American Journal of Obstetrics and Gynecology*, **148**, 939–945.

Kuwana, T. and Fujimoto, T. (1983) Active locomotion of human primordial germ cells *in vitro*. *Anatomical Record*, **205**, 21–26.

La Marca, M.J. and Wassarman, P.M. (1979) Programme of early development in the mammal: Changes in absolute rates of synthesis of ribosomal proteins during oogenesis and early embryogenesis in the mouse. *Developmental Biology*, **73**, 103–119.

Lambertsen, C.J., Greenbaum, D.F., Wright, K.H. and Wallach, E.E. (1976) *In vitro* studies of ovulation in the perfused rabbit ovary. *Fertility and Sterility*, **27**, 178–187.

Lamprecht, S.A., Zor, U., Tsafriri, A. and Lindner, H.R. (1973) Action of PGE_2 and of LH on ovarian adenylate cyclase, protein kinase and ornithine decarboxylase activity during postnatal development and maturity in the rat. *Journal of Endocrinology*, **57**, 217–233.

Larsen, J.K., Byskov, A.G. and Christensen, I.J. (1986) Flow cytometry and sorting of meiotic prophase cells of female rabbits. *Journal of Reproduction and Fertility*, **76**, 587–596.

Larsen, W.J., Wert, S.E. and Brunner, G.D. (1986) A dramatic loss of cumulus cell gap junctions is correlated with germinal vesicle breakdown in rat oocytes. *Developmental Biology*, **113**, 517–521.

Laufer, N., Reich, R., Braw, R, Shenker, J.G. and Tsafriri, A. (1982) The effect of clomiphene citrate on preovulatory rat follicles in culture. *Biology of Reproduction*, **27**, 463–471.

Lawrence, T.S., Dekel N. and Beers, W.H. (1980) Binding of human chorionic gonadotropin by rat cumuli oophori and granulosa cells: A comparative study. *Endocrinology*, **106**, 1114–1118.

Lee, W. and Novy, M.J. (1978) Effects of luteinizing hormone and indomethacin on blood flow and steroidogenesis in the rabbit ovary. *Biology of Reproduction*, **18**, 799–807.

Leibfried, L. and First, N.L. (1980a) Follicular control of meiosis in the porcine oocyte. *Biology of Reproduction*, **23**, 705–709.

Leibfried, L. and First, N.L. (1980b) Effect of bovine and porcine follicular fluid and granulosa cells on maturation of oocytes *in vitro*. *Biology of Reproduction*, **23**, 699–704.

LeMaire, W.J. (1989) Mechanism of mammalian ovulation. *Steroids*, **54**, 455–469.

LeMaire, W.J. and Marsh, J.M. (1975) Interrelationships between prostaglandins, cyclic AMP and steroids in ovulation. *Journal of Reproduction and Fertility*, Suppl. 22, 53–74.

LeMaire, W.J., Yang, N.S.T., Behrman, H.H., Marsh, J.M. (1973) Preovulatory changes in the concentration of prostaglandins in rabbit Graafian follicles. *Prostaglandins*, **3**, 367–376.

LeMaire, W.J., Janson, P.O., Källfelt, B.J., Holmes, P.V., Cajander, S., Bjersing, L. and Ahrén, K. (1982) The preovulatory decline in follicular estradiol is not required for ovulation in the rabbit. *Acta Endocrinologica*, **101**, 452–457.

Li, X.M., Sagawa, N., Ihara, Y., Okagaki, A., Hasegawa, M., Inamori, K., Itoh, H., Mori, T. and Ban, C. (1991) The involvement of platelet-activating factor in thrombocytopenia and follicular rupture during gonadotropin-induced superovulation in immature rats. *Endocrinology*, **129**, 3132–3138.

Lieberman, M.E., Barnea, A., Bauminger, S., Tsafriri, A., Collins, W.P. and Lindner, H.R. (1975) LH effect on the pattern of steroidogenesis in cultured Graafian follicles of the rat: Dependence on macromolecular synthesis. *Endocrinology*, **96**, 1533–1542.

Lieberman, M.E., Tsafriri, A., Bauminger, S., Collins, W.P., Ahrén, K. and Lindner, H.R. (1976) Oocyte meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinologica*, **83**, 151–157.

Lima-de-Faria, A. and Borum, K. (1962) The period of DNA synthesis prior to meiosis in the mouse. *Journal of Cell Biology*, **14**, 381–388.

Lin, T-H., Ukeshima, A., Maeyama, M. and Fujimoto, T. (1982) Scanning electron microscopic study on human primordial germ cells during the migration period. *Okajimas Folia Anatomica Japonica*, **59**, 305–320.

Lindner, H.R., Tsafriri, A., Lieberman, M.E., Zor, U., Koch, Y., Bauminger, S. and Barnea, A. (1974) Gonadotropin action on cultured Graafian follicles: Induction of maturation

division of the mammalian oocyte and differentiation of the luteal cell. *Recent Progress in Hormone Research*, **30**, 79–138.

Lindner, H.R., Bar-Ami, S. and Tsafriri, A. (1980) Model systems for studying oocyte maturation in *Animal Models in Human Reproduction*, (eds M. Serio and L. Martini), Raven Press, New York, pp. 65–85.

Lintern-Moore, S. and Moore, G.P.M. (1979) The initiation of follicle and oocyte growth in the mouse ovary. *Biology of Reproduction*, **20**, 773–778.

Lipner, H. (1973) Mechanism of mammalian ovulation in *Handbook of Physiology, Endocrinology*, Vol. II, Pt I (ed. R. Greep), American Physiological Society, Washington DC, pp. 409–437.

Lipner, H. (1988) Mechanism of mammalian ovulation in *The Physiology of Reproduction*, (eds E. Knobil and J.D. Neil), Raven Press, New York, pp. 447–488.

Lipner, H. and Greep, R.O. (1971) Inhibition of steroidogenesis at various sites in the biosynthetic pathway in relation to induced ovulation. *Endocrinology*, **88**, 602–607.

Lipner, H. and Smith, M.S., (1971) A method for determining the distribution and source of protein in preovulatory rat ovaries. *Journal of Endocrinology*, **50**, 1–14.

Lipner, H. and Wendelken, L. (1971) Inhibition of ovulation by inhibition of steroidogenesis in immature rats 35447. *Proceedings of the Society for Experimental Biology and Medicine*, **136**, 1141–1145.

Lischinsky, A. and Armstrong, D.T. (1983) Granulosa cell stimulation of thecal androgen synthesis. *Canadian Journal of Physiology and Pharmacology*, **61**, 472–477.

Liu, Y.X., Ny, T., Sarkar, D., Loskutoff, D., Hsueh, A.J.W. (1986) Identification and regulation of tissue plasminogen activator activity in rat cumulus–oocyte complexes. *Endocrinology*, **119**, 1578–1587.

Liu, Y.X., Peng, X.R. and Tor, N.Y. (1991) Tissue-specific and time-coordinated hormone regulation of plasminogen-activator-inhibitor type-1 and tissue-type plasminogen activator in the rat ovary during gonadotropin-induced ovulation. *European Journal of Biochemistry*, 195, 549–555.

Loewenstein, W.R. (1981) Junctional intercellular communication: The cell-to-cell membrane channel. *Physiological Reviews*, **61**, 829–913.

Lohka, M.J., Hayes, M.K. and Maller, J.L. (1988) Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proceedings of the National Academy of Sciences of the USA*, **85**, 3009–3013.

Louvet, J.P. and Vaitukaitus, J.L. (1976) Induction of FSH receptors in rat ovaries by estrogen priming. *Endocrinology*, **99**, 758–764.

Luciani, J.M., Devictor-Vuillet, M., Gagne, R. and Stahl, A. (1974) An air-drying method for first meiotic prophase preparations from mammalian ovaries. *Journal of Reproduction and Fertility*, **36**, 409–411.

Macdonald, C. (1985) Gap junctions and cell–cell communication. *Essays in Biochemistry*, **21**, 86–118.

McGaughey, R.W. (1983) Regulation of oocyte maturation. *Oxford Review of Reproduction and Biology*, **5**, 106–130.

McGaughey, R.W. and Van Blerkom, J. (1977) Patterns of polypeptide synthesis of porcine oocytes during maturation *in vitro*. *Developmental Biology*, **56**, 241–254.

McKay, D.C., Hertig, A.T., Adams, E.C. and Danziger, S. (1953) Histochemical observations on the germ cells of human embryos. *Anatomical Record*, **117**, 201–219.

McLaren, A. (1981) *Germ Cell and Soma: A New Look at an Old Problem*, Yale University Press, New Haven, Connecticut.

McLaren, A. (1983a) Primordial germ cells in mice in *Problems of the Keimbahn*, (ed. W. Hilscher), Karger, Basle, pp. 59–66.

McLaren, A. (1983b) Does the chromosomal sex of a mouse germ cell affect its development? in *Current Problems in Germ Cell Differentiation*, (eds A. McLaren and C.C. Wylie), Cambridge University Press, pp. 225–240.

McLaren, A. (1984) Meiosis and differentiation of mouse germ cells in *Controlling Events in Meiosis*, (eds C.W. Evans and H.G. Dickinson), Symposia of the Society for Experimental Biology, Symposium XXXVIII, The Company of Biologists Ltd, Cambridge, pp. 25–43.

McLaren, A. and Monk, M. (1981) X chromosome activity in the germ cells of sex-reversed mouse embryos. *Journal of Reproduction and Fertility*, **63**, 533–537.

McNatty, K.P. (1981) Hormonal correlates of follicular development in the human ovary. *Austrian Journal of Biological Science*, **34**, 249–268.

McNatty, K.P. and Baird, D.T. (1978) Relationship between follicle-stimulating hormone, androste-

nedione and oestradiol in human follicular fluid. *Journal of Endocrinology*, **76**, 527–531.

McNatty, K.P., Baird, D.T., Bolton, A., Chambers, P., Corker, C.S. and McLean, H. (1976) Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. *Journal of Endocrinology*, **71**, 77–85.

McNatty, K.P., Makris, A., DeGrazier, C., Osathanondh, R. and Ryan, K.J. (1979a) The production of progesterone, androgens and estrogens by granulosa cells, thecal tissue and stromal tissue from human ovaries *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **49**, 687–699.

McNatty, K.P., Makris, A., DeGrazia, C. and Ryan, K.J. (1979b) Production of progesterone, androgens and oestrogens by human granulosa cells *in vitro* and *in vivo*. *Journal of Steroid Biochemistry*, **11**, 775–779.

McNatty, K.P., Moore-Smith, D., Osathanondh, R. and Ryan, K.J. (1979c) The human antral follicle: functional correlates of growth and atresia. *Annales de Biologie Animales, Biochimie et Biophysique*, **19**, 1547–1558.

McNatty, K.P., Moore-Smith, D., Makris, A., Osathanondh, R. and Ryan, K.J. (1979d) The microenvironment of the human antral follicle; interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte *in vivo* and *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **49**, 851–860.

McNatty, K.P., Gibb, M., Dobson, C., Ball, K., Coster, J., Heath, D. and Thurley, D.C. (1982) Preovulatory follicular development in sheep treated with PMSG and/or prostaglandin. *Journal of Reproduction and Fertility*, **65**, 111–123.

McNatty, K.P., Hillier, S.G., Van den Boogard, A.M.J., Trimbos-Kemper, T.C.M., Reichert Jr., L.E. and Van Hall, E.V. (1983) Follicular development during the luteal phase of the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, **56**, 1022–1031.

Magnusson, C., Hillensjö, T., Tsafriri, A., Hultborn, R. and Ahrén, K. (1977) Oxygen consumption of maturing rat oocytes. *Biology of Reproduction*, **17**, 9–15.

Maia Jr., H., Barbosa, I. and Coutinho, E.M. (1978) Inhibition of ovulation in marmoset monkeys by indomethacin. *Fertility and Sterility*, **29**, 565–570.

Makris, A. and Ryan, K.J. (1975) Progesterone, androstenedione, testosterone, estrone and estradiol synthesis in hamster ovarian follicle cells. *Endocrinology*, **96**, 694–701.

Makris, A. and Ryan, K.J. (1980) The source of follicular androgens in the hamster follicle. *Steroids*, **35**, 53–64.

Maller, J.L. (1985) Oocyte maturation in amphibians in *Developmental Biology*, Vol. 1 (ed. L.W. Browder), Plenum Publishing Corporation, New York, pp. 289–311.

Mangia, F. and Canipari, R. (1977) Biochemistry of growth and maturation in mammalian oocytes in *Development in Mammals*, Vol. 2, (ed. M.H. Johnson), Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands, pp. 1–29.

Mangia, F. and Epstein, C.J. (1975) Biochemical studies of growing mouse oocytes: preparation of oocytes and analysis of glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities. *Developmental Biology*, **45**, 211–220.

Mangia, F., Erickson, R.P. and Epstein, C.J. (1976) Synthesis of LDH-1 during mammalian oogenesis and early development. *Developmental Biology*, **54**, 146–150.

Mann, J.S., Kindy, M.S., Edwards, D.R. and Curry, J.T.E. (1991) Hormonal regulation of matrix metalloproteinase inhibitors in rat granulosa cells and ovaries. *Endocrinology*, **128**, 1825–1832.

Maro, B., Johnson, M.H., Webb, M. and Flach, G. (1986) Mechanism of polar body formation in the mouse oocyte: On interaction between the chromosomes, the cytoskeleton and the plasma membrane. *Journal of Embryology and Experimental Morphology*, **92**, 11–32.

Martinat, N. and Combarnous, Y. (1983) The release of plasminogen activator by rat granulosa cells is highly specific for FSH activity. *Endocrinology*, **113**, 433–435.

Marut, E.L., Huang, S.C. and Hodgen, G.D. (1983) Distinguishing the steroidogenic roles of granulosa and theca cells of the dominant ovarian follicle and corpus luteum. *Journal of Clinical Endocrinology and Metabolism*, **57**, 925–930.

Masui, Y. and Clarke, H.J. (1979) Oocyte maturation. *International Reviews of Cytology*, **57**, 185–282.

Mattioli, M., Geleati, G. and Seren, E. (1986) Pig oocyte maturation *in vitro* and evaluation of their developmental capability by IVF. *Atti della Societta Italiana delle Science Veterinarie*, **40**, 200–202.

Mattioli, M., Geleati, G. and Seren, E. (1988a)

Effect of follicle somatic cells during pig oocyte maturation on egg penetrability and male pronucleus formation. *Gamete Research*, **20**, 177–183.

Mattioli, M., Geleati, G., Bacci, M.L. and Seren, E. (1988b) Follicular factors influence oocyte fertilizability by modulating the intercellular cooperation between cumulus cells and oocyte. *Gamete Research*, **21**, 223–232.

Mauléon, P. (1967) Cinétiques de l'ovogenese chez le mamiferes. *Archives of Anatomical Microscopy and Morphology*, **56**, 125–150.

Mauléon, P. (1969) Oogenesis and folliculogenesis in *Reproduction in Domestic Animals*, (ed. H.H. Cole), Academic Press, New York, pp. 187–215.

Mauléon, P. and Mariana, J.C. (1976) Oogenesis and folliculogenesis in *Reproduction in Domestic Animals*, (eds H.H. Cole and P.T. Cupps), Academic Press, New York, pp. 275–302.

Mauléon, P. and Reviers, M.M. (1969) Variations avec liage et le sexe des teneurs en hormones folliculo-stimulante (FSH) et luteisante (LH) des adenohypophyses de foetus de mouton. *Annales de Biologie Animale, Biochimie et Biophysique*, **9**, 475–481.

Mazur, M. and YoungLai, E.V. (1986) Role of the pituitary in controlling oogenesis in the rabbit. *Biology of Reproduction*, **35**, 191–197.

Meinecke, B. and Meinecke-Tillman, S. (1981) Induction and inhibition of meiotic maturation of follicle-enclosed porcine oocytes *in vivo*. *Theriogenology*, **216**, 205–209.

Merchant-Larios, H., (1975) Rat gonadal and ovarian organogenesis with and without germ cells. An ultrastructural study. *Developmental Biology*, **44**, 1–21.

Merchant-Larios, H. (1976) The role of germ cells in the morphogenesis and cytodifferentiation of the rat ovary in *Progress in Differentiation Research*, (ed. N. Muller-Bérat), North/Holland, Amsterdam, pp. 453–462.

Merchant-Larios, H. (1978) Ovarian differentiation in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 47–81.

Merchant-Larios, H. and Coello, J. (1979) The effect of busulfan on rat primordial germ cells at the ultrastructural level. *Cell Differentiation*, **8**, 145–155.

Merk, F.B., Albright, J.T. and Botticelli, C.R. (1973) The fine structure of granulosa cell nexuses in rat ovarian follicles. *Anatomical Record*, **175**, 107–126.

Miller, J.G.O. and Behrman, H.R. (1986) Oocyte maturation is inhibited by adenosine in the presence of FSH. *Biology of Reproduction*, **35**, 833–837.

Miller, W.A. and Jagiello, G. (1973) Gonadotropin dependency of isolated ovine ovarian follicles cultured 24 h *in vitro*. *Fertility and Sterility* **24**, 609–617.

Mintz, B. (1959) Continuity of the female germ cell line from embryo to adult. *Archives of Anatomical Microscopy and Morphology*, **48**, 155–172.

Mintz, B. and Russell, E.S. (1957) Gene induced embryological modification of primordial germ cells in the mouse. *Journal of Experimental Zoology*, **134**, 207–230.

Mitchell, P.A. and Burghardt, R.C. (1986) The ontogeny of nexuses (gap junctions) in the ovary of the fetal mouse. *Anatomical Record*, **214**, 283–288.

Miyayama, Y., Fuyuta, M. and Fujimoto, T. (1977) Ultrastructural observations on the origin, amoeboid features and associations of human primordial germ cells. *Acta Anatomia Nippon*, **52**, 255–268.

Monk, M. and McLaren, A. (1981) X chromosome activity in foetal germ cells of the mouse. *Journal of Embryology and Experimental Morphology*, **63**, 75–84.

Moor, R.M. (1977) Sites of steroid production in ovine Graafian follicles in culture. *Journal of Endocrinology*, **73**, 143–150.

Moor, R.M. (1978) Role of steroids in the maturation of ovine oocytes. *Annales de Biologie Animale, Biochimie et Biophysique*, **18**, 477–482.

Moor, R.M. and Crosby, I.M. (1986) Protein requirements for germinal vesicle breakdown in ovine oocytes. *Journal of Embryology and Experimental Morphology*, **94**, 207–220.

Moor, R.M. and Heslop, J.P. (1981) Cyclic AMP in mammalian follicle cells and oocytes during maturation. *Journal of Experimental Zoology*, **216**, 205–209.

Moor, R.M. and Osborn, J.C. (1983) Somatic control of protein synthesis in mammalian oocytes during maturation in *Molecular Biology of Egg Maturation*, (eds R. Porter, and J. Whelan), Ciba Foundation Symposium, No. 7, Pitman Press, London, pp. 178–196.

Moor, R.M. and Trounson, A.O. (1977) Hormonal and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity. *Journal of Reproduction and Fertility*, **49**, 101–109.

Moor, R.M., Smith, M.W. and Dawson, R.M.C. (1980a) Measurement of intercellular coupling between oocytes and cumulus cells using intra-

cellular markers. *Experimental Cell Research*, **126**, 15–30.

Moor, R.M., Polge, C. and Willadsen, S.M. (1980b) Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. *Journal of Embryology and Experimental Morphology*, **56**, 319–335.

Moor, R.M., Osborn, J.C., Cran, D.G. and Walters, D.E. (1981) Selective effect of gonadotrophins on cell coupling, nuclear maturation and protein synthesis in mammalian oocytes. *Journal of Embryology and Experimental Morphology*, **61**, 347–365.

Moore, G.P.M. (1978) RNA synthesis in fixed cells by endogenous RNA polymerases. *Experimental Cell Research*, **111**, 317–326.

Moore, G.P.M. and Lintern-Moore, S. (1978) Transcription of the mouse oocyte genome. *Biology of Reproduction*, **18**, 865–870.

Morales, T.I., Woessner, J.F., Howell, D.S., Marsh, J.M. and LeMaire, W.J. (1978) A microassay for the direct demonstration of collagenolytic activity in Graafian follicles of the rat. *Biochimica et Biophysica Acta*, **524**, 428–434.

Morales, T.I., Woessner, J.F., Marsh, J.M. and LeMaire, W.J. (1983) Collagen, collagenase and collagenolytic activity in rat Graafian follicles during follicular growth and ovulation. *Biochimica et Biophysica Acta*, **756**, 119–122.

Mori, T., Suzuki, A., Nishimura, T. and Kambegawa, A. (1977a) Inhibition of ovulation in immature rats by anti-progesterone antiserum. *Journal of Endocrinology*, **73**, 185–186.

Mori, T., Suzuki, A., Nishimura, T. and Kambegawa, A. (1977b) Evidence for androgen participation in induced ovulation in immature rats. *Endocrinology*, **101**, 623–626.

Morioka, N., Brännström, M., Koos, R.D. and LeMaire, W.J. (1988) Ovulation in the perfused ovary *in vitro*: Further evidence that estrogen is not required. *Steroids*, **51**, 173–183.

Morishima, A. and Grumbach, M.M. (1968) The interrelationship of sex chromosome constitution and phenotype in the syndrome of gonadal diogenesis and its variants. *Annals of the New York Academy of Science*, **155**, 695–715.

Motlik, J. and Fulka, J. (1976) Breakdown of the germinal vesicle in pig oocytes *in vivo* and *in vitro*. *Journal of Experimental Zoology*, **198**, 155–162.

Motlik, J. and Fulka, J. (1981) Fertilization and rabbit oocytes co-cultured with granulosa cells. *Journal of Reproduction and Fertility*, **63**, 425–429.

Motlik, J. and Fulka, J. (1986) Factors affecting meiotic competence in pig oocytes. *Theriogenology*, **25**, 87–96.

Motlik, J., Crozet, N. and Fulka, J. (1984a) Meiotic competence *in vitro* of pig oocytes isolated from early antral follicles. *Journal of Reproduction and Fertility*, **72**, 323–328.

Motlik, J., Kopecny, V., Travnik, P. and Pivko, J. (1984b) RNA synthesis in pig follicular oocytes. Autoradiographic and cytochemical study. *Biologie Cellulaire*, **50**, 229–236.

Motlik, J., Fulka, J. and Fléchon, J.E. (1986) Changes in intercellular coupling between pig oocytes and cumulus cells during maturation *in vivo* and *in vitro*. *Journal of Reproduction and Fertility*, **76**, 31–37.

Müller, W.P. (1974) The lampbrush chromosomes of *Xenopus laevis* (Daudin). *Chromosoma*, **47**, 283–296.

Murdoch, W.J. (1985) Follicular determinants of ovulation in the ewe. *Domestic Animal Endocrinology*, **2**, 105–121.

Murdoch, W.J., Nix, K.J. and Dunn, T.G. (1983) Dynamics of ovarian blood supply to periovulatory follicles of the ewe. *Biology of Reproduction*, **28**, 1001–1006.

Murdoch, W.J., Peterson, T.A. Van Kirk, E.A. Vincent, D.L. and Inskeep, E.K. (1986) Interactive roles of progesterone, prostaglandins and collagenase in the ovulatory mechanism of the ewe. *Biology of Reproduction*, **35**, 1187–1194.

Murray, A.W. and Kirschner, M.W. (1989) Cyclin synthesis drives the early embryonic cell cycle. *Nature*, **339**, 275–280.

Murray, A.W., Solomon, M.J. and Kirschner, M.W. (1989) The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature*, **339**, 280–286.

Nakano, R., Mizuno, T. Katayama, K. and Tojo, S. (1975) Growth of ovarian follicles in rats in the absence of gonadotrophins. *Journal of Reproduction and Fertility*, **45**, 545–546.

Neal, P. and Baker, T.G. (1973) Response of mouse ovaries *in vivo* and in organ culture to pregnant mare's serum gonadotrophin and human chorionic gonadotrophin. I. Examination of critical time intervals. *Journal of Reproduction and Fertility*, **33**, 339–410.

Nicosia, S.V. and Mikhail, G. (1975) Cumuli oophori in tissue culture: Hormone production, ultrastructure, and morphometry of early luteinization. *Fertility and Sterility*, **26**, 427–448.

Nicosia, S.V., Wolf, D.P. and Inoue, M. (1977) Cortical granule distribution and cell surface

characteristics in mouse eggs. *Developmental Biology*, **57**, 56–74.

Nilsson, L., Wikland, M. and Hamberger, L. (1982) Recruitment of an ovulatory follicle in the human following follicle-ectomy and luteectomy. *Fertility and Sterility*, **37**, 30–34.

Nimrod, A., Erickson, G.F. and Ryan, K.J. (1976) A specific FSH receptor in rat granulosa cells: properties of binding *in vitro*. *Endocrinology*, **98**, 56–64.

Nuti, L.C., McShan, W.H. and Meyer, R.K. (1974) Effect of ovine FSH and LH on serum steroids and ovulation in hypophysectomized immature female rats. *Endocrinology*, **95**, 682–689.

Ny, T., Elgh, F. and Lund, B. (1984) The structure of the human tissue-type plasminogen activator gene: Correlation of intron and exon structures to functional and structural domains. *Proceedings of the National Academy of Sciences of the USA*, **81**, 5355–5359.

Ny, T., Bjersing, L., Hsueh, A.J.W. and Loskutoff, D.J. (1985) Cultured granulosa cells produce two plasminogen activators and an antiactivator, each regulated differently by gonadotropins. *Endocrinology*, **116**, 1666–1668.

O, W.S. and Baker, T.G. (1976) Initiation and control of meiosis in hamster gonads *in vitro*. *Journal of Reproduction and Fertility*, **48**, 399–401.

O, W.S., Robertson, D.M. and de Kretser, D.M. (1989) Inhibin as an oocyte meiotic inhibitor. *Molecular and Cellular Endocrinology*, **62**, 307–311.

Oakberg, E.F. (1968) Relationship between stage of follicular development and RNA synthesis in the mouse oocyte. *Mutation Research*, **6**, 155–165.

O'Grady, J.P., Caldwell, B.V., Auletta, F.J. and Speroff, L. (1972) The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy and pseudopregnancy in the rabbit. *Prostaglandins*, **1**, 97–106.

Ohno, S. and Smith, J.B. (1964) Role of fetal follicular cells in meiosis and mammalian oocytes. *Cytogenetics*, **3**, 324–333.

Ohno, S., Kaplan, W.D. and Kinosita, R. (1961) X-chromosome behaviour in germ and somatic cells of *Rattus norvegicus*. *Experimental Cell Research*, **22**, 535–544.

Ohno, S., Klinger, H.P. and Atkin, N.B. (1962) Human oogenesis. *Cytogenetics*, **1**, 42–51.

Olds, P.J., Stern, S. and Biggers, J.D. (1973) Chemical estimates of the RNA and DNA contents of the early mouse embryo. *Journal of Experimental Zoology*, **186**, 39–46.

Olsiewski, P.J. and Beers, W.H. (1983) cAMP synthesis in the rat oocyte. *Developmental Biology*, **100**, 287–293.

Orczyk, G.P. and Behrman, H.R. (1972) Ovulation blockade by aspirin or indomethacin – *in vivo* evidence for a role of prostaglandin in gonadotropin secretion. *Prostaglandins*, **1**, 3–20.

Osborn, J.C. and Moor, R.M. (1983a) Time-dependent effects of α-amanitin on nuclear maturation and protein synthesis in mammalian oocytes. *Journal of Embryology and Experimental Morphology*, **73**, 317–338.

Osborn, J.C. and Moor, R.M. (1983b) The role of steroid signals in the maturation of mammalian oocytes. *Journal of Steroid Biochemistry*, **19**, 133–137.

Osborn, J.C., Moor, R.M. and Crosby, I.M. (1986) Effect of alterations in follicular steroidogenesis on the nuclear and cytoplasmic maturation of ovine oocytes. *Journal of Embryology and Experimental Morphology*, **98**, 187–208.

Ozdzenski, W. (1967) Observations on the origin of primordial germ cells in the mouse. *Zoologica Poloniae*, **17**, 367–379.

Parr, E.L. (1974) Histological examination of the rat ovarian follicle wall prior to ovulation. *Biology of Reproduction*, **11**, 483–503.

Parr, E.L. (1975) Rupture of ovarian follicles at ovulation. *Journal of Reproduction and Fertility*, Suppl. 22, 1–22.

Parvinen, M., Byskov, A.G., Yding Andersen, C. and Grinsted, J. (1982) Is the spermatogonia cycle regulated by MIS and MPS? *Annals of the New York Academy of Sciences*, **383**, 483–488.

Patrono, C. (1983) Arachidonic acid metabolism in the ovary: Biochemistry, methodology and physiology in *Comprehensive Endocrinology – The Ovary*, (ed. G.B. Serra), Raven Press, New York, pp. 45–56.

Payne, R.W. and Runser, R.H. (1959) Quantitative response of the rat ovary to pituitary gonadotrophin as modified by estrogen. *Endocrinology*, **65**, 383–394.

Pedersen, H. and Seidel Jr., G. (1972) Micropapillae; a local modification of the cell surface observed in rabbit oocytes and adjacent follicle cells. *Journal of Ultrastructural Research*, **39**, 540–548.

Pedersen, T. (1970) Follicle kinetics in the ovary of the cyclic mouse. *Acta Endocrinologica*, **64**, 304–323.

Pedersen, T. (1972) Follicle growth in the mouse ovary in *Oogenesis*, (eds J.D. Biggers and A.W.

Schuetz), University Park Press, Baltimore, pp. 261–276.

Pedersen, T. and Peters, H. (1968) Proposal for a classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility*, **17**, 555–557.

Pelliniemi, I.J., Dym, M. (1980) The fetal gonad and sexual differentiation in *Maternal–Fetal Endocrinology*, (eds D. Tulchinsky and K.J. Ryan), Saunders, Philadelphia, PA, pp. 252–280.

Pelliniemi, I.J. and Lauteala, L. (1981) Development of sexual dimorphism in the embryonic gonad. *Human Genetics*, **58**, 64–67.

Peters, H. (1970) Migration of gonocytes into the mammalian gonad and their differentiation. *Philosophical Transactions of the Royal Society of London Series B*, **9**, 91–101.

Peters, H. (1978) Folliculogenesis in mammals in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 121–144.

Peters, H. and Crone, M. (1967) DNA synthesis in oocytes of mammals. *Archives d'Anatomie Microscopique et de Morphologie Experimentale*, **56**, Suppl. 3-4, 1601–70.

Peters, H. and McNatty, K.P. (1980) *The Ovary*, Granada Publishing, London.

Peters, H., Levy, E. and Crone, M. (1965) Oogenesis in rabbits. *Journal of Experimental Zoology*, **185**, 169–180.

Peters, H., Byskov, A.G., Himelstein-Braw, R. and Faber, M. (1975) Follicular growth: the basic event in the mouse and human ovary. *Journal of Reproduction and Fertility*, **45**, 559–566.

Peters, H., Himelstein-Braw, R. and Faber, M. (1976) The normal development of the ovary in childhood. *Acta Endocrinologica*, **82**, 617–630.

Peters, H., Byskov, A.G. and Grinsted, J. (1978) Follicular growth in fetal and prepubertal ovaries of human and other primates in *Gynaecological Endocrinology*, (eds G.T. Ross and M.B. Lipsett), W.B. Saunders & Co, London, pp. 469–485.

Peterson, C.M., Zhu, C., Mukaida, T., Butler, T.A., Woessner, J.F. and LeMarie, W.J. (1993) The angiotensin II antagonist, saralasin inhibits ovulation in the perfused rat ovary. *American Journal of Obstetrics and Gynecology*, **168**, 242–245.

Phillips, D.M. and Shalgi, R. (1980) Surface architecture of the mouse and hamster zona pellucida and oocyte. *Journal of Ultrastructural Research*, **72**, 1–12.

Phillips, D.M., Shalgi, R., Kraicer, P.F. and Segal, S.J. (1978) The rat oocyte–cumulus complex during ovulation and fertilization as seen with the SEM in *Scanning Electron Microscopy*, Vol. II, (ed. A.M.S. O'Hare), SEM Inc., Illinois, pp. 1113–1122.

Picard, J.Y. and Josso, N. (1984) Purification of testicular anti-Müllerian hormone allowing direct visualization of the pure glycoprotein and determination of yield and purification factor. *Molecular and Cellular Endocrinology*, **34**, 23–29.

Picard, J.Y., Goulut, C. Bourrillon, R. and Josso, N. (1986) Biochemical analysis of bovine testicular anti-Müllerian hormone, *Federation of European Biochemical Societies Letters*, **195**, 73–76.

Piko, L. and Clegg, K.B. (1982) Quantitative changes in total RNA, total poly(a) and ribosomes in early mouse embryos. *Developmental Biology*, **89**, 362–378.

Pincus, G. and Enzmann, E.V. (1935) The comparative behaviour of mammalian eggs *in vivo* and *in vitro*. *Journal of Experimental Medicine*, **62**, 655–675.

Pincus, G. and Enzmann, E.V. (1937) The growth, maturation and atresia of ovarian eggs in the rabbit. *Journal of Morphology*, **61**, 351–383.

Piper, P.J. (ed.) (1983) *Leukotrienes and Other Lipoxygenase Products*, Research Studies Press (A division of John Wiley & Sons), Chichester.

Polani, P.E. and Crolla, J.A. (1981) An experimental approach to meiosis in the female mouse in *Development and Function of Reproductive Organs*, (eds A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 338–348.

Polani, P.E. and Crolla, J.A. (1989) An experimental test of the production line hypothesis of oogenesis in the female mouse in *Evolutionary Mechanisms in Sex Determination*, (ed. S.S. Wachtel), CRC Press Inc., Boca Raton, FL.

Politis, I., Srikandakumar, J., Turner, J.D., Tsang, B.K., Ainsworth, L. and Downey, B.R. (1990) Changes in and partial identification of the plasminogen activator and plasminogen activator inhibitor systems during ovarian follicular maturation in the pig. *Biology of Reproduction*, **43**, 636–642.

Pomerantz, S.H., Channing, C.P. and Tsafriri, A. (1979) Studies on the purification and action of an oocyte maturation inhibitor isolated from porcine follicular fluid in *Peptides, Structure and Biological Function*, (eds E. Gross and J. Meinhofer), Pierce Chemical Company, Rockford, Illinois, pp. 765–774.

Pomerantz, S.H., Bilelo, P.A. and Evans, V. (1989) Recent developments in the purification of an

inhibitor of oocyte maturation from pig follicular fluid in *Follicular Development and the Ovulatory Response*, (eds A. Tsafriri and N. Dekel), Serono Symposia Review No. 23, Rome, pp. 77–86.

Presl, J., Pospisil, J., Figarova, V. and Wagner, V. (1972) Developmental changes in uptake of radioactivity by the ovaries, pituitary and uterus after ^{125}I-labelled human chorionic gonadotrophin administration in rats. *Journal of Endocrinology*, **52**, 585–586.

Presl, J., Propsisil, J., Figarova, V. and Krabec, Z. (1974) Stage-dependent changes in binding of iodinated FSH during ovarian follicle maturation in rats. *Endocrinologica Experimentalis (Bratisl)*, **8**, 291–298.

Purandare, T.V., Munshi, S.R. and Rao, S.S. (1976) Effect of antisera to gonadotropins on follicular development and fertility of mice. *Biology of Reproduction*, **15**, 311–320.

Rabinovici, J., Blankestein, J. Goldman, B., Rudak, E., Dor, Y., Pariente, C., Geier, A., Lunenfeld, B. and Mashiach, S. (1988) *In vitro* fertilization and primary embryonic cleavage are possible in 17α-hydroxylase deficiency despite extremely low intrafollicular 17β-estradiol. *Journal of Clincal Endocrinology and Metabolism*, **68**, 693–699.

Racowsky, C. (1983) Androgenic modulation of cyclic adenosine monophosphate (cAMP)-dependent meiotic arrest. *Biology of Reproduction*, **28**, 774–787.

Racowsky, C. (1984) Effect of forskolin on the spontaneous maturation and cyclic AMP content of rat oocyte–cumulus complexes. *Journal of Reproduction and Fertility*, **72**, 107–116.

Racowsky, C. (1985a) Effect of forskolin on maintenance of meiotic arrest and stimulation of cumulus expansion, progesterone, and cyclic AMP production by pig oocyte–cumulus complexes. *Journal of Reproduction and Fertility*, **74**, 9–21.

Racowsky, C. (1985b) Antagonistic actions of estradiol and tamoxifen upon forskolin-dependent meiotic arrest, intercellular coupling, and the cyclic AMP content of hamster oocyte–cumulus complexes. *Journal of Experimental Zoology*, **234**, 251–261.

Racowsky, C. (1985c) Effect of forskolin on the spontaneous maturation and cyclic AMP content of hamster oocyte–cumulus complexes. *Journal of Experimental Zoology*, **234**, 87–96.

Racowsky, C. (1991) Gamete resources: Origin and production of oocytes. In *Animal Applications of Research in Mammalian Development* (eds R.A. Pedersen, A. McLaren and N.L. First), Cold Spring Harbor Laboratory Press, pp. 23–82.

Racowsky, C. and Baldwin, K.V. (1989) *In vitro* and *in vivo* studies reveal that hamster oocyte meiotic arrest is maintained only transiently by follicular fluid, but persistently by membrane-/cumulus granulosa cell contact. *Developmental Biology*, **134**, 297–306.

Racowsky, C. and McGaughey, R.W. (1982a) Further studies of the effects of follicular fluid and membrana granulosa cells on the spontaneous maturation of pig oocytes. *Journal of Reproduction and Fertility*, **66**, 505–512.

Racowsky, C. and McGaughey, R.W. (1982b) In the absence of protein, estradiol suppresses meiosis of porcine oocytes *in vitro*. *Journal of Experimental Zoology*, **224**, 103–110.

Racowsky, C. and Satterlie, R.A. (1985) Metabolic, fluorescent dye and electrical coupling between hamster oocytes and cumulus cells during meiotic maturation *in vivo* and *in vitro*. *Developmental Biology*, **60**, 318–325.

Racowsky, C., Baldwin, K.V., Larabell, C.A., DeMarais, A.A. and Kazilek, C.J. (1989) Down-regulation of membrana granulosa gap junctions is correlated with irreversible commitment to resume meiosis in golden Syrian hamster oocytes. *European Journal of Cell Biology*, **49**, 244–251.

Rao, M.C., Richards, J.S., Midgley Jr., A.R. and Reichert Jr., L.E. (1977) Regulation of gonadotropin receptors by luteinizing hormone in granulosa cells. *Endocrinology*, **101**, 512–523.

Rasmussen, S.W. and Holm, P.B. (1980) Mechanics of meiosis. *Hereditas*, **93**, 187–216.

Rawson, J.M.R. and Espey, L.L. (1977) Concentration of electron dense granules in the rabbit ovarian surface epithelium during ovulation. *Biology of Reproduction*, **17**, 561–566.

Reddoch, R.B., Pelletier, R.M., Barbe, G.J. and Armstrong, D.T. (1986) Lack of ovarian responsiveness to gonadotropic hormones in infantile rats sterilized with busulfan. *Endocrinology*, **119**, 879–886.

Reich, R., Laufer, N., Lewysohn, O., Cordova, T., Ayalon, D. and Tsafriri, A. (1982) *In vitro* effects of cannabinoids on follicular function in the rat. *Biology of Reproduction*, **27**, 223–231.

Reich, R., Kohen, F., Zaor, Z. and Tsafriri, A. (1983) Possible involvement of lipoxygenase products of arachidonic acid pathway in ovulation. *Prostaglandins*, **26**, 1011–1020.

Reich, R., Miskin, R. and Tsafriri, A. (1985a) Follicular plasminogen activator: Involvement in ovulation. *Endocrinology*, **116**, 516–521.

Reich, R., Tsafriri, A. and Mechanic, G.L. (1985b) The involvement of collagenolysis in ovulation in the rat. *Endocrinology*, **116**, 522–527.

Reich, R., Kohen, F., Slager, R. and Tsafriri, A. (1985c) Ovarian lipoxygenase activity and its regulation by gonadotropin in the rat. *Prostaglandins*, **30**, 581–590.

Reich, R., Miskin, R. and Tsafriri, A. (1986) Intrafollicular distribution of plasminogen activators and their hormonal regulation *in vitro*. *Endocrinology*, **119**, 1588–1601.

Reich, R., Haberman, S., Abisogun, A.O., Sofer, Y., Grossman, S., Adelmann-Grill, B.C. and Tsafriri, A. (1987) Follicular rupture at ovulation: Collagenase activity and metabolism of arachidonic acid in *The Control of Follicle Development, Ovarian and Luteal Function: Lessons from In Vitro Fertilization* (eds F. Naftolin and A.H. deCherney) Raven Press, New York, pp. 317–329.

Reich, R., Daphna-Iken, D., Chun, S.Y., Popliker, M., Slager, R., Adelmann-Grill, B.C. and Tsafriri, A. (1991) Preovulatory changes in ovarian expression of collagenases and tissue metalloproteinase inhibitor mRNA: Role of eicosanoids. *Endocrinology*, **129**, 1869–1875.

Reichert Jr., L.E. (1962) Endocrine influences on rat ovarian proteinase activity. *Endocrinology*, **70**, 697–700.

Reinthaller, A., Bieglmayer, C., Kirchheimer, J.C., Christ, G., Deutinger, J. and Binder, B.R. (1990) Plasminogen activators, plasminogen activator inhibitor and fibronectin in human granulosa cells and follicular fluid related to oocyte maturation and intrafollicular gonadotropin levels. *Fertility and Sterility*, **54**, 1045–1051.

Riccio, A., Grimaldi, G., Verde, P., Gebastio, G., Boast S., Blasi, F. (1985) The human urokinase plasminogen activator gene and its promoter. *Nucleic Acids Research*, **13**, 2759–2771.

Rice, C. and McGaughey, R.W. (1981) Effect of testosterone and dibutyryl cAMP on the spontaneous maturation of pig oocytes. *Journal of Reproduction and Fertility*, **62**, 245–256.

Richards, J.S. (1975) Estradiol receptor content in rat granulosa cells during follicular development: Modification by estradiol and gonadotropins. *Endocrinology*, **97**, 1174–1184.

Richards, J.S. (1978) Hormonal control of follicular growth and maturation in mammals in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 331–360.

Richards, J.S. (1980) Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiological Reviews*, **60**, 51–89.

Richards, J.S. and Bogovich, K. (1982) Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. *Endocrinology*, **111**, 1429–1438.

Richards, J.S., Ireland, J.J., Rao, M.C., Bernath, G.A., Midgley Jr., A.R. and Reichert Jr., L.E. (1976) Ovarian follicular development in the rat: Hormone receptor regulation by estradiol, follicle stimulating hormone and luteinizing hormone. *Endocrinology*, **99**, 1562–1570.

Richter, J.D. and McGaughey, R.W. (1979) Specificity of inhibition by steroids of porcine oocyte maturation *in vitro*. *Journal of Experimental Zoology*, **209**, 81–90.

Richter, J.D. and McGaughey, R.W. (1981) Patterns of polypeptide synthesis in mouse oocytes during germinal vesicle breakdown and during maintenance of the germinal vesicle stage by dibutyryl cAMP. *Developmental Biology*, **83**, 188–192.

Rime, H., Neant, I., Guerrier, P. and Ozon, R. (1989) 6-Dimethylaminopurine (6-DMAP), a reversible inhibitor of the transition to metaphase during the first meiotic cell division of the mouse oocyte. *Developmental Biology*, **133**, 169–179.

Rodman, T.C. and Bachvarova, R. (1976) RNA synthesis in preovulatory mouse oocytes. *Journal of Cell Biology*, **70**, 251–257.

Rogulska, A.R., Ozdzenski, W. and Komar, A. (1971) Behaviour of mouse primordial germ cells in the chick embryo. *Journal of Embryology and Experimental Morphology*, **25**, 115–264.

Rom, E., Reich, R., Laufer, N., Lewin, A., Rabinowitz, R., Pevsner, B., Lancet, M., Shenker, J.G., Miskin, R., Adelmann-Grill, B. and Tsafriri, A. (1987) Follicular fluid contents as predictors of success of *in vitro* fertilization – embryo transfer. *Human Reproduction*, **2**, 505–510.

Rondell, P. (1964) Follicular pressure and distensibility in ovulation. *American Journal of Physiology*, **207**, 590–594.

Rondell, P. (1970a) Biophysical aspects of ovulation. *Biology of Reproduction*, Suppl. 2, 64–89.

Rondell, P. (1970b) Follicular processes in ovulation. *Federation Proceedings*, **29**, 1875–1879.

Ross, G.T. and Lipsett, M.B. (1978) Hormonal cor-

relates of normal and abnormal follicle growth after puberty in humans and other primates. *Clinical and Endocrinological Metabolism*, **7**, 561–575.

Rüsse, I. (1982) Interzellularbrücken zwischen weiblichen Keimzellen bei Rind u. Schaf. *Verhandlungen Anatomischer Gesellschaft*, **76**, 367–369.

Rüsse, I. (1983) Oogenesis in cattle and sheep. *Bibliotheca Anatomica*, **24**, 77–92.

Ruby, J.R., Dyer, R.F. and Skalko, R.G. (1969) The occurrence of intercellular bridges during oogenesis in the mouse. *Journal of Morphology*, **127**, 307–340.

Ryan, K.J. (1979) Granulosa-thecal cell interaction in ovarian steroidogenesis. *Journal of Steroid Biochemistry*, **11**, 799–800.

Ryan, K. (1981) Follicular atresia: Some speculations of biochemical markers and mechanisms in *Dynamics of Ovarian Function*, (eds N.B. Schwartz and M. Hunzicker-Dunn), Raven Press, New York, pp. 1–11.

Salder, S.E. and Maller, J.L. (1981) Progesterone inhibits adenylate cyclase in *Xenopus* oocytes. Action on the guanine nucleotide regulatory protein. *Journal of Biological Chemistry*, **256**, 6368–6373.

Sadler, S.E. and Maller, J.L. (1982) Identification of a steroid receptor on the surface of *Xenopus* oocytes by photoaffinity labelling. *Journal of Biological Chemistry*, **257**, 355–361.

Salustri, A. and Martinozzi, M. (1983) A comparison of protein synthetic activity in *in vitro* cultured denuded and follicle-enclosed oocytes. *Cell Biology International Reports*, **7**, 1049–1055.

Salustri, A. and Siracusa, G. (1983) Metabolic coupling, cumulus expansion and meiotic resumption in mouse cumuli oophori cultured *in vitro* in the presence of FSH or dcAMP, or stimulated *in vivo* by hCG. *Journal of Reproduction and Fertility*, **68**, 335–341.

Sato, E. and Ishibashi, T. (1977) Meiotic arresting action of the substance obtained from cell surface of porcine ovarian granulosa cells. *Japanese Journal of Zootechnological Science*, **48**, 22–26.

Sato, E. and Koide, S.S. (1984) A factor from bovine granulosa cells preventing oocyte maturation. *Differentiation*, **26**, 59–62.

Sato, E., Furubayashi, R. and Ishibashi, T. (1978) Physico-chemical characteristics of the meiosis arresting substance obtained from cell surface of porcine granulosa cells. *Japanese Journal of Animal Reproduction*, **24**, 59–62.

Sato, E., Wood, H.N., Lynn, D.G. and Koide, S.S. (1985) Modulation of oocyte maturation by cyclic adenosine 3,5-pyrophosphate. *Cell Differentiation*, **17**, 179–184.

Satoh, K., Fukuoko, H., Wu, T., Mitsuhashi, N., Kinoshita, K. and Sakamoto, S. (1981) Inhibition of superovulation in immature female rats by the antiserum to cyclooxygenase. *Prostaglandins and Medicine*, **7**, 29–41.

Schochet, S.S. (1916) A suggestion as to the process of ovulation and ovarian cyst formation. *Anatomical Record*, **10**, 447–457.

Schroeder, A.C. and Eppig, J.J. (1984) The developmental capacity of mouse oocytes that matured spontaneously *in vitro* is normal. *Developmental Biology*, **102**, 493–497.

Schroeder, A.C. and Eppig, J.J. (1989) Developmental capacity of mouse oocytes that undergo maturation *in vitro*: effect of the hormonal state of the oocyte donor. *Gamete Research*, **24**, 81–92.

Schuetz, A.W. (1974) Role of hormones in oocyte maturation. *Biology of Reproduction*, **10**, 150–178.

Schuetz, A.W. (1985) Local control mechanisms during oogenesis and folliculogenesis in *Developmental Biology. A Comprehensive Synthesis*, Vol. I, *Oogenesis*, (ed. L.W. Browder), Plenum Press, New York, pp. 3–83.

Schuetz, A.W. and Rock, J. (1982) Stimulatory and inhibitory effects of human follicular fluid on amphibian oocyte maturation and ovulation *in vitro*. *Differentiation*, **21**, 41–44.

Schultz, R.M. (1985) Roles of cell-to-cell communication in development. *Biology of Reproduction*, **32**, 27–42.

Schultz, R.M. (1989) Molecular aspects of mammalian oocyte growth and maturation in *Experimental Approaches to Mammalian Embryonic Development* (eds J. Rossand and R. Pederson), Cambridge University Press, Cambridge, UK.

Schultz, R.M. (1991) Meiotic maturation of mammalian oocytes. In *Elements of Mammalian Fertilization*, Vol. I (ed. P.M. Wassarman), CRC Press, Boca Raton, Ann Arbor, Boston, pp. 77–104.

Schultz, R.M., Wassarman, P.M. (1977a) Specific changes in the pattern of protein synthesis during meiotic maturation of mammalian oocytes *in vitro*. *Proceedings of the National Academy of Sciences of the USA*, **74**, 538–541.

Schultz, R.M., Wassarman, P.M. (1977b) Biochemical studies of mammalian oogenesis: Protein synthesis during oocyte growth and meiotic maturation in the mouse. *Journal of Cell*

Science, **24**, 167–194.

Schultz, R.M., LaMarca, M.J. and Wassarman, P.M. (1978) Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes *in vitro*. *Proceedings of the National Academy of Sciences of the USA*, **75**, 4160–4164.

Schultz, R.M., Letorneau, G.E. and Wassarman, P.M. (1979a) Program of early development in the mammal: changes in the patterns and absolute rates of tubulin and total protein synthesis during oogenesis and early embryogenesis in the mouse. *Developmental Biology*, **68**, 341–359.

Schultz, R.M., Letourneau, G.E. and Wassarman, P.M. (1979b) Program of early development in the mammal: Changes in the patterns and absolute rates of tubulin and total protein synthesis during oocyte growth in the mouse. *Developmental Biology*, **73**, 120–133.

Schultz, R.M., Montgomery, R.R. and Belanoff, J.R. (1983a) Regulation of mouse oocyte meiotic maturation: Implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. *Developmental Biology*, **97**, 264–273.

Schultz, R.M., Montgomery, R.R., Ward-Bailey, P.F. and Eppig, J.J. (1983b) Regulation of oocyte maturation in the mouse: Possible roles of intercellular communication, cAMP, and testosterone. *Developmental Biology*, **95**, 294–304.

Schwartz, N.B. and Channing, C.P. (1977) Evidence for ovarian inhibin: Suppression of the secondary rise in serum follicle stimulating hormone levels in proestrous rats by injection of porcine follicular fluid. *Proceedings of National Academy of Sciences of the USA*, **74**, 5721–5724.

Schwartz, N.B., Krone, K., Talley, W.L. and Ely, C.A. (1973) Administration of antiserum to ovine FSH in the female rat: Failure to influence immediate events of cycle. *Endocrinology*, **92**, 1165–1174.

Schwartz, N.B., Cobbs, S.B., Talley, W.L. and Ely, C.A. (1975) Induction of ovulation by LH and FSH in the presence of antigonadotrophic sera. *Endocrinology*, **96**, 1171–1178.

Schwarzel, W.C., Kruggel, W.G. and Brodie, H.J. (1973) Studies on the mechanism of estrogen biosynthesis. VIII. The development of inhibitors of the enzyme system in human placenta. *Endocrinology*, **92**, 866–880.

Seamark, R.F., Moor, R.M. and McIntosh, J.E.A. (1974) Steroid hormone production by sheep ovarian follicles cultured *in vitro*. *Journal of Reproduction and Fertility*, **41**, 143–158.

Sellens, M.H. and Jenkinson, E.J. (1975) Permeability of the mouse zona pellucida to immunoglobulin. *Journal of Reproduction and Fertility*, **42**, 153–157.

Sheela Rani, C.S. and Moudgal, N.R. (1977) Role of proestrus surge of gonadotropins in the initiation of follicular maturation in the cyclic hamster: a study using antisera to follicle stimulating hormone and luteinizing hormone. *Endocrinology*, **101**, 1484–1494.

Sherizly, I., Galiani, D. and Dekel, N. (1988) Regulation of oocyte maturation: communication in the rat cumulus–oocyte complex. *Human Reproduction*, **3**, 761–766.

Shimada, H., Okamura, H., Noda, Y., Suzuki, A., Tojo, S. and Takada, A. (1983) Plasminogen activator in rat ovary during the ovulatory process: independence of prostaglandin mediation. *Journal of Endocrinology*, **97**, 201–205.

Shimzie, S., Masahiro, T. and Dean, J. (1983) *In vitro* biosynthesis of three sulfated glycoproteins of murine zona pellucida by oocytes grown in follicle culture. *Journal of Biological Chemistry*, **258**, 5858–5863.

Singh, R.P. and Carr, D.H. (1966) The anatomy and histology of XO human embryos and fetuses. *Anatomical Record*, **155**, 369–384.

Siracusa, G., De Felici, M. and Salustri, A. (1985) The proliferative and meiotic history of mammalian female germ cells in *Biology of Fertilization*, Vol. I (eds C.B. Metz and A. Monroy), Academic Press, Orlando, pp. 253–297.

Sirad, M.A. and First, N.L. (1988) *In vitro* inhibition of oocyte nuclear maturation in the bovine. *Biology of Reproduction*, **39**, 229–234.

Sirad, M.A. and Bilodeau, S. (1990a) Granulosa cells inhibit the resumption of meiosis in bovine oocytes *in vitro*. *Biology of Reproduction*, **43**, 777–783.

Sirad, M.A. and Bilodeau, S. (1990b) Effects of granulosa cell co-culture on *in vitro* meiotic resumption of bovine oocytes. *Journal of Reproduction and Fertility*, **89**, 459–465.

Smith, D.M. and Tenney, D.Y. (1979) Effect of hypophysectomy on mouse oocyte maturation *in vitro*. *Journal of Reproduction and Fertility*, **55**, 415–422.

Smokovits, A., Kokolia, N. and Alexaki-Tzivanidu, E. (1988) The plasminogen activator activity is markedly increased mainly at the area of the rupture of the follicular wall at the time of ovulation. *Animal Reproduction Science*, **16**, 285–294.

Snow, M.H.L. and Monk, M. (1983) Emergence and migration of mouse primordial germ cells in *Current Problems in Germ Cell Differentiation*, (eds A. McLaren and C.C. Wylie), Cambridge University Press, Cambridge. pp. 115–135.

Snyder, B.W., Beecham, G.D. and Schane, H.P. (1984) Inhibition of ovulation in rats with epostane, an inhibitor of 3β-hydroxysteroid dehydrogenase (41865). *Proceedings of the Society for Experimental Biology and Medicine*, **176**, 238–242.

Sorensen, R.A. and Wassarman, P.M. (1976) Relationship between growth and meiotic maturation of mouse oocyte. *Developmental Biology*, **50**, 531–536.

Sorensen, R.A., Cyert, M.S. and Pedersen, R.A. (1985) Active maturation promoting factor is present in mature mouse oocytes. *Journal of Cellular Biology*, **100**, 1637–1640.

Speed, R.M. (1982) Meiosis in the foetal mouse ovary. I. An analysis at the light microscope level using surface-spreading. *Chromosoma*, **85**, 427–437.

Spiegelman, H. and Bennett, D. (1973) A light- and electron-microscope study of primordial germ cells in the early mouse embryo. *Journal of Embryology and Experimental Morphology*, **30**, 97–118.

Stahl, A., Gagne, R., Devictor-Vuillet, M., Luciani, J.M. and Vagner-Capodano, A.M. (1973) Association d'ARN a un corpuscule chromatinien au cours de la prophase meiotique de l'ovocyte foetal humain. *Comptes Rendus de l'Academie des Sciences*, **277**, 1549–1552.

Stahl, A., Luciani, J.M., Devictor, M., Vagner-Capodano, A.M. and Gagne, R. (1975) Constitutive heterochromatin and micronucleoli in the human oocyte at the diplotene stage. *Humangenetik*, **26**, 315–327.

Staigmiller, R.B. and Moor, R.M. (1984) Effect of follicle cells on the maturation and developmental-competence of ovine oocytes mature outside the follicle. *Gamete Research*, **9**, 221–229.

Stein, I.E. and Anderson, C.H. (1979) A qualitative and quantitative study of rete ovarii development in the fetal rat: correlation with the onset of meiosis and follicle cell appearance. *Anatomical Record*, **193**, 197–211.

Sternlicht, A.L. and Schultz, R.M. (1981) Biochemical studies of mammalian oogenesis: Kinetics of accumulation of total and poly (a)-containing RNA during growth of the mouse oocyte. *Journal of Experimental Zoology*, **215**, 191–200.

Stoklosowa, S., Gregoraszczuk, E. and Channing, C.P. (1982) Estrogen and progesterone secretion by isolated cultured porcine thecal and granulosa cells. *Biology of Reproduction*, **26**, 943–952.

Stone, S.L., Pomerantz, S.H., Schwartz-Kripner, A. and Channing, C.P. (1978) Inhibitor of oocyte maturation from porcine follicular fluid: further purification and evidence for reversible action. *Biology of Reproduction*, **19**, 585–592.

Stouffer, R.L., Aladin Chandrasekher, Y., Brenner, R.M., Hild-Petito, S.A., Melner, M.H. and Slayden, O.D. (1993) *Expression of sex steroid receptors in the primate ovary*. Proceedings 75th Annual Meeting of the Endocrine Society, Las Vegas, June 9–12, The Endocrine Society Press, Bethesda, Maryland.

Strickland, S. and Beers, W.H. (1976) Studies on the role of plasminogen activation in ovulation. *Journal of Biological Chemistry*, **251**, 5694–5702.

Suenson, E., Lützen, O. and Thorsen, S. (1984) Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. *European Journal of Biochemistry*, **140**, 513–522.

Swanson, R.J. and Lipner, H. (1977) Mechanism of ovulation: Effect of intrafollicular progesterone antiserum in rabbits. *Federation Proceedings*, **36**, 390.

Szöllösi, D., (1972) Changes in Some Cell Organelles During Oogenesis in Mammals, *Oogenesis*, (eds J.D. Biggers and A.W. Schuetz), University Park Press, Baltimore. pp. 47–64.

Szöllösi, D. (1975) Ultrastructural Aspects of Oocyte Maturation and Fertilization in Mammals, (ed. C. Thibault), La Fécondation, Masson, Paris, pp. 13–35.

Szöllösi, D., Gérard, M., Ménézo, Y. and Thibault, C. (1978) Permeability of ovarian follicle, corona cell–oocyte relationship in mammals. *Annales de Biologie Animale, Biochemie Biophysique*, **18**, 511–521.

Szybek, K. (1972) *In vitro* maturation of oocytes from sexually immature mice. *Journal of Endocrinology*, **54**, 527–528.

Takahashi, M., Ford, J.J., Yoshinaga, K. and Greep, R.O. (1974) Induction of ovulation in hypophysectomized rats by progesterone. *Endocrinology*, **95**, 1322–1326.

Takahashi, M., Koide, S.S. and Donohue, P.K. (1986) Müllerian inhibiting substance as oocyte

meiosis inhibitor. *Molecular and Cellular Endocrinology*, **47**, 225–234.

Tam, P.P.L. and Snow, M.H.L. (1981) Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *Journal of Embryology and Experimental Morphology*, **64**, 135–147.

Tarkowski, A.K. (1959) Experiments on the developments of isolated blastomeres of mouse eggs. *Nature*, **188**, 1286–1287.

Tarkowski, A.K. (1966) An air-drying method for chromosome preparations from mouse eggs. *Cytogenetics*, **5**, 394–400.

Tarkowski, A.K. and Balakier, H. (1980) Nucleo-cytoplasmic interactions in cell hybrids between mouse oocytes, blastomeres and somatic cells. *Journal of Embryology and Experimental Morphology*, **55**, 922–927.

Tate, J.R. (1986) Coordinated assembly of the developing egg. *BioEssays*, **4**, 197–201.

Templeton, A.A., Messinis, I.E. and Baird, D.T. (1986) The characteristics of ovarian follicles in spontaneous and stimulated cycles in which there was an endogenous LH surge. *Fertility and Sterility*, **46**, 1113–1117.

Thibault, C. (1972) Final Stages of Mammalian Oocyte Maturation, in *Oogenesis*, (eds J.D. Biggers and A.W. Schuetz), University Park Press, Baltimore, pp. 397–411.

Thibault, C. (1977) Are follicular maturation and oocyte maturation independent processes? *Journal of Reproduction and Fertility*, **51**, 1–15.

Thibault, C. and Gérard, M. (1973) Cytoplasmic and nuclear maturation of rabbit oocytes *in vitro*. *Annales de Biologie Animale, Biochimie et Biophysique*, **13**, 145–155.

Thibault, C. and Levasseur, M.C. (1988) Ovulation. *Human Reproduction*, **3**, 513–523.

Thibault, C., Gérard, M. and Menezo, Y. (1975a) Preovulatory and ovulatory mechanisms in oocyte maturation. *Journal of Reproduction and Fertility*, **45**, 605–610.

Thibault, C., Gérard, M. and Menezo, Y. (1975b) Acquisition par l'ovocyte de Lapine et de Veau du facteur de decondensation du noyau du spermatozoide fécondant (MPGF). *Annales de Biologie Animale, Biochimie et Biophysique*, 705–714.

Thibault, C., Gérard, M. and Menezo, Y. (1976) Nuclear and cytoplasmic aspects of mammalian oocyte maturation *in vitro* in relation to follicle size and fertilization in *Progress in Reproductive Biology 1*, (ed. P.O. Hubinout), S. Karger Press, Basle, pp. 233–240.

Thibault, C., Szöllösi, D. and Gérard, M. (1987) Mammalian oocyte maturation. *Reproduction, Nutrition and Development*, **27**, 865–896.

Thompson, E.A. and Siiteri, P.K. (1974) The involvement of human placental microsomal cytochrome p-450 in aromatization. *Journal of Biological Chemistry*, **249**, 5373–5378.

Törnell, J., Brännström, M., Chari, S. and Hillensjö, T. (1984) Synergistic inhibitory effects by human follicular fluid and cAMP on spontaneous rat oocyte maturation. *Biology of Reproduction*, **30**, Suppl. 1, Abstract 28.

Törnell, J., Billig, H. and Hillensjö, T. (1991) Regulation of oocyte maturation by changes in ovarian levels of cyclic nucleotides. *Human Reproduction*, **6**, 411–422.

Tonetta, S.A. and diZerega, G.S. (1986) Paracrine regulation of follicular maturation in primates. *Clinical Endocrinology and Metabolism*, **15**, 135.

Too, C.K.L., Bryant-Greenwood, G.D. and Greenwood, F.C. (1984) Relaxin increases the release of plasminogen activator, collagenase and proteoglycanase from rat granulosa cells *in vitro*. *Endocrinology*, **115**, 1043–1050.

Tsafriri, A. (1978) Oocyte maturation in mammals in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum Press, New York, pp. 409–433.

Tsafriri, A. (1979) Mammalian oocyte maturation: Model systems and their physiological relevance in *Ovarian Follicular and Corpus Luteum Function*, (eds C.P. Channing, J.J. Marsh and W.A. Sadler), Plenum Press, New York, pp. 269–281.

Tsafriri, A. (1985) The control of meiotic maturation in mammals in *Biology of Fertilization*, Vol. 1, (eds C.B. Metz and A. Monroy), Academic Press, New York, pp. 221–252.

Tsafriri, A. and Bar-Ami, S. (1982) Oocyte maturation inhibitor: A 1981 perspective in *Intraovarian Mechanisms*, (eds C.P. Channing and S.J. Segal), Plenum Press, New York, pp. 145–149.

Tsafriri, A. and Braw, R. (1984) Experimental approaches to atresia in mammals. *Oxford Review of Reproduction and Biology*, **6**, 226–265.

Tsafriri, A. and Channing, C.P. (1975a) Influence of follicular maturation and culture conditions on the meiosis of pig oocytes *in vitro*. *Journal of Reproduction and Fertility*, **43**, 149–152.

Tsafriri, A. and Channing, C.P. (1975b) An inhibitory influence of granulosa cells and follicular fluid upon porcine oocyte meiosis *in vitro*. *Endocrinology*, **96**, 922–927.

Tsafriri, A. and Dekel, N. (1993) Molecular mechanisms in ovulation in *Cellular and Molecular Mechanisms in Female Reproduction* (ed. J.K. Findlay), Academic Press, Inc., New York, (in press).

Tsafriri, A. and Pomerantz, S.H. (1984) The control of ovum maturation in mammals: Comparative aspects in *Gonadal Peptides and Their Biological Significance*, (eds M.R. Sairam and L.E. Atkinson), World Scientific Publishing Company, Singapore, pp. 191–206.

Tsafriri, A., Lindner, H.R., Zor, U. and Lamprecht, S.A. (1972a) *In vitro* induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. *Journal of Reproduction and Fertility*, **31**, 39–50.

Tsafriri, A., Lindner, H.R., Zor, U. and Lamprecht, S.A. (1972b) Physiological role of prostaglandins in the induction of ovulation. *Prostaglandins*, **2**, 1–10.

Tsafriri, A., Lieberman, M.E., Barnea, A., Bauminger, S. and Lindner, H.R. (1973) Induction by LH of ovum maturation and of steroidogenesis in isolated Graafian follicles of the rat: Role of RNA and protein synthesis. *Endocrinology*, **93**, 1378–1386.

Tsafriri, A., Lieberman, M.E., Koch, Y., Bauminger, S., Chobsieng, P., Zor, U. and Lindner, H.R. (1976a) Capacity of immunologically purified FSH to stimulate cyclic AMP accumulation and steroidogenesis in Graafian follicles and to induce ovum maturation and ovulation in the rat. *Endocrinology*, **98**, 655–661.

Tsafriri, A., Lieberman, M.E., Ahrén, K. and Lindner, H.R. (1976b) Dissociation between LH-induced aerobic glycolysis and oocyte maturation in cultured Graafian follicles of the rat. *Acta Endocrinologica*, **81**, 362–366.

Tsafriri, A., Pomerantz, S.H. and Channing, C.P. (1976c) Follicular control of oocyte maturation in *Ovulation in the Human*, (eds P.G. Crosignani and D.R. Mishell), Academic Press, New York, pp. 31–39.

Tsafriri, A., Pomerantz, S.H. and Channing, C.P. (1976d) Inhibition of oocyte maturation by porcine follicular fluid: Partial characterization of the inhibitor. *Biology of Reproduction*, **14**, 511–516.

Tsafriri, A., Channing, C.P., Pomerantz, S.H. and Lindner, H.R. (1977) Inhibition of maturation of isolated rat oocytes by porcine follicular fluid. *Journal of Endocrinology*, **75**, 285–291.

Tsafriri, A., Abisogun, A.O. and Reich, R. (1987) Steroids and follicular rupture at ovulation. *Journal of Steroid Biochemistry*, **27**, 359–363.

Tsafriri, A., Picard, J.Y. and Josso, N. (1988) Immunopurified anti-Müllerian hormone does not inhibit spontaneous maturation *in vitro* of rat oocytes. *Biology of Reproduction*, **38**, 481–485.

Tsafriri, A., Vale, W. and Hsueh, A.J.W. (1989a) Effects of transforming growth factors and inhibin-related proteins on rat preovulatory Graafian follicles *in vitro*. *Endocrinology*, **125**, 1857–1862.

Tsafriri, A., Bicsak, T.A., Cajander, S.B., Ny, T. and Hsueh, J.W. (1989b) Suppression of ovulation rate by antibodies to tissue-type plasminogen activator and α_2-antiplasmin. *Endocrinology*, **124**, 415–421.

Tsafriri, A., Daphna-Iken, D. and Abisogun, A.O. (1989c) Vascular factors and ovulation in *Development and Function of the Reproductive Organs*, Vol. III, (ed. N. Josso), Ares-Serono Symposia, Rome, pp. 249–257.

Tsafriri, A., Daphna-Iken, D., Abisogun, A.O. and Reich, R. (1991a) Follicular rupture during ovulation: Activation of collagenolysis. In *Advances in Assisted Reproductive Technologies* (eds S. Mashiach, Z. Ben-Rafael, N. Laufer and J.G. Schenker) Plenum Press, New York, pp. 103–112.

Tsafriri, A., Veljkovic, M.V., Pomerantz, S.H. and Ling, N. (1991b) The action of transforming growth factors and inhibin-related proteins on oocyte maturation. *Bulletin de L'Association des Anatomistes*, **75**, 109–113.

Tsafriri, A., Chung, S.Y. and Reich, R. (1993) The involvement of 5-lipoxygenase products in follicular rupture during ovulation. *American Endocrine Society*, 1993, Abstract.

Tsuda, H. (1965) An electron microscope study on the oogenesis in the mouse, with special reference to the behaviours of oogonia and oocytes at meiotic prophase. *Archives of Histology Japan*, **25**, 533–555.

Tsuji, K., Sowa, M. and Nakano, R. (1985) Relationship between human oocyte maturation and different follicular sizes. *Biology of Reproduction*, **32**, 413–417.

Turnbull, K.E., Braden, A.W.H. and Mattner, P.E. (1977) The pattern of follicular growth and atresia in the bovine ovary. *Australian Journal of Biological Science*, **30**, 229–241.

Ueno, S., Manganaro, T.F. and Donahoe, P.K. (1988) Human recombinant Müllerian inhibiting substance inhibition of rat oocyte meiosis is

reversed by epidermal growth hormone *in vitro*. *Endocrinology*, **123**, 1652–1659.

Upadhyay, S. and Zamboni, L. (1982) Ectopic germ cells. A natural model for the study of germ cell sexual differentiation. *Proceedings of the National Academy of Sciences of the USA*, **79**, 6584–6588.

Urner, F. and Schorderet-Slatkine, S. (1984) Inhibition of denuded mouse oocyte meiotic maturation by tumor-promoting phorbol esters and its reversal by retinoids. *Experimental Cell Research*, **154**, 600–605.

Urner, F., Herrmann, W.L., Baulieu, E.E. and Schorderet-Slatkine, S. (1983) Inhibition of denuded mouse oocyte meiotic maturation by forskolin, an activator of adenylate cyclase. *Endocrinology*, **113**, 1170–1172.

Van Blerkom, J. (1980) Cell and molecular biology of meiotic maturation of the mammalian oocyte in *Biology of the Ovary*, (eds P.M. Motta and E.S.E. Hafex), Martinus Nijhoff, The Hague, pp. 179–190.

Van Blerkom, J. (1981) Structural relationship and post-translational modification of stage-specific proteins synthesized during early pre-implantation development in the mouse. *Proceedings of the National Academy of Sciences of the USA*, **78**, 7629–7633.

Van Blerkom, J. (1985) Protein synthesis during oogenesis and early embryogenesis in the mammal in *Biology of Fertilization*, Vol. 3, (eds C.B. Metz and A. Monroy), Academic Press, Orlando, pp. 379–399.

Van Blerkom, J. and Bell, H. (1986) Regulation of development in the fully grown mouse oocyte: Chromosome-mediated temporal and spatial differentiation of the cytoplasm and plasma membrane. *Journal of Embryology and Experimental Morphology*, **93**, 213–218.

Van Blerkom, J. and McGaughey, R.W. (1978) Molecular differentiation of the rabbit ovum. I. During oocyte maturation *in vivo* and *in vitro*. *Developmental Biology*, **63**, 139–150.

Van Blerkom, J. and Motta, P.M. (1979) *The Cellular Basis of Mammalian Reproduction*. Urban & Schwarzenberg, Baltimore.

Van Blerkom, J. and Runner, M.N. (1984) Mitochondrial reorganization during resumption of arrested meiosis in the mouse oocyte. *American Journal of Anatomy*, **171**, 335–355.

Vassali, J.D., Sappino, A.P. and Belin, D. (1991) The plasminogen activator/plasmin system. *Journal of Clinical Investigations*, **88**, 1067–1072.

Vermande-van Eck, G.J. (1956) Neo-ovogenesis in the adult monkey. *Anatomical Record*, **125**, 207–224.

Vermeiden, J.P.W. and Zeilmaker, G.H. (1974) Relationship between maturation, division, ovulation and luteinization in the female rat. *Endocrinology*, **95**, 341–351.

Vernon, M.W., Dierschke, D.J., Sholl, S.A. and Wolf, R.C. (1983) Ovarian aromatase activity in granulosa and theca cells of Rhesus monkeys. *Biology of Reproduction*, **28**, 342–349.

Vivarelli, E., Conti, M., DeFelici, M. and Siracusa, G. (1983) Meiotic resumption and intracellular cAMP levels in mouse oocytes treated with compounds which act on cAMP metabolism. *Cell Differentiation*, **12**, 271–276.

van Wagenen, G. and Simpson, M.E. (1965) *Embryology of the Ovary and Testis Homo Sapiens and Macaca mulatta*, Yale University Press, New Haven.

Wallach, E.E., Bronson, R., Hamada Y., Wright, K.H. and Stevens, V.C. (1975) Effectiveness of $PGF_{2\alpha}$ in restoration of HMG-HCG induced ovulation in indomethacin-treated rhesus monkeys. *Prostaglandins*, **10**, 129–138.

Wallach, E.E., Bronson, R.A., Kobayashi, Y., Hamada, Y. and Wright, K.H. (1980) The physiology of ovulation in *Endocrine Physiopathology of the Ovary*, (eds R.I. Tozzini, G. Reeves and R.L. Pineda), Elsevier/North Holland Biomedical Press, Amsterdam, pp. 153–163.

Wang, C. and Leung, A. (1983) Gonadotropin regulate plasminogen activator production by rat granulosa cells. *Endocrinology*, **112**, 1201–1207.

Wang, C. and Leung, A. (1989) Glucocorticoids stimulate plasminogen activator production by rat granulosa cells. *Endocrinology*, **124**, 1595–1601.

Warnes, G.M., Moor, R.M. and Johnson, M.H. (1977) Changes in protein synthesis during maturation of sheep oocytes *in vivo* and *in vitro*. *Journal of Reproduction and Fertility*, **49**, 331–335.

Wartenberg, H. (1983) Structural aspects of gonadal differentiation in mammals and birds in *Differentiation*, (eds U. Muller and W.W. Franke), Springer-Verlag, Berlin, pp. 64–71.

Wassarman, P. (1983) Oogenesis: Synthetic events in the developing mammalian egg in *Mechanism and Control of Animal Fertilization*, (ed. J. Hartman), Academic Press, New York, pp. 1–54.

Wassarman, P. (1988) The mammalian ovum. In

Physiology of Reproduction (eds. E. Knobil and J. Neill), Raven Press, New York, pp. 69–102.

Wassarman, P.M. and Josefowicz, W.J. (1978) Oocyte development in the mouse: an ultrastructural comparison of oocytes isolated at various stages of growth and meiotic competence. *Journal of Morphology*, **156**, 209–236.

Wassarman, P.M. and Kinloch, R.A. (1992) Gene expression during oogenesis in mice. *Mutation Research*, **296**, 3–15.

Wassarman, P.M. and Letourneau, G.E. (1976) RNA synthesis in fully-grown mouse oocytes. *Nature*, **261**, 73–74.

Wassarman, P.M. and Mrozak, S.C. (1981) Program of early development in the mammal: Synthesis and intracellular migration of histone H4 during oogenesis in the mouse. *Developmental Biology*, **84**, 364–371.

Wassarman, P.M., Florman, H.M. and Greeve, J.M. (1985) Receptor medicated sperm-egg interactions in mammals in *Biology of Fertilization*, Vol. 2, (eds C.B. Metz and A. Monroy), Academic Press, Orlando, pp. 341–360.

Wassarman, W.J. and Smith, D.L. (1978) Oocyte maturation in non-mammalian vertebrates in *The Vertebrate Ovary*, (ed. R.E. Jones), Plenum, New York, pp. 443–468.

Weakley, B.S. (1967) Light and electron microscopy of developing germ cells and follicle cells in the ovary of the golden hamster; twenty-four hours before birth to eight days postpartum. *Journal of Anatomy*, **101**, 435–459.

Weakley, B.S. (1968) Comparison of cytoplasmic lamellae and membranous elements in the oocytes of five mammalian species. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **85**, 109–123.

Webb, R. and Gauld, I.K. (1985) Folliculogenesis in sheep: control of ovulation rate in *Genetics of Reproduction in Sheep*, (eds R.B. Land and D.W. Robinson), Butterworth, London, pp. 261–274.

Weimer, S.L., Campeau, J.D., Marrs, R.P. and DiZerega, G.S. (1984) Alteration of human follicular fluid plasminogen activator activity by ovarian hyperstimulation. *Journal of In Vitro Fertilization and Embryo Transfer*, **1**, 263–266.

Weir, B.J. and Rowlands, J.W. (1977) Ovulation and atresia in *The Ovary*, 2nd edn, Vol. 1, (eds S Zuckerman and B.J. Weir), Academic Press, New York, pp. 265–301.

Weiss, L. (1971) Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries in Turner's syndrome. *Journal of Medical Genetics*, **8**, 540–544.

van de Wiel, D.F.M., Bar-Ami, S., Tsafriri, A. and de Jong, F.H. (1983) Oocyte maturation inhibitor, inhibin, and steroid concentrations in porcine follicular fluid at various stages of the oestrous cycle. *Journal of Reproduction and Fertility*, **68**, 247–252.

von Winiwarter, H. (1901) Recherches sur l'ovogenese et l'organogenese de l'ovaire des mammiferes (lapin et homme). *Archives de Biologie*, **17**, 33–199.

von Winiwarter, H. and Sainmont, G. (1909) Nouvelle recherches sur l'ovogénèse et l'organogénèse de l'ovaire de mammifères (chat). *Archives de Biologie*, **24**, 1–142.

Witschi, E. (1948) Migration of the germ cells of human embryos from the yolksac to the primitive gonadal fold. *Contributions to Embryology*, **32**, 67–80.

Woessner Jr., J.F., Butler, T., LeMaire, W.J., Morioka, N., Mukaida, T. and Zhu, C. (1989) The role of collagenase in ovulation in the rat in *Follicular Development and the Ovulatory Response*, (eds A. Tsafriri and N. Dekel), Ares-Serono Symposia, Rome, pp. 169–178.

Wolgemuth, D.J. and Gavin, B.J. (1981) Ultrastructural and biochemical characterization of gene expression in follicular oocytes in neonatal and prepubertal rats in *Development and Function of Reproductive Organs*, (eds A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 289–298.

Wolgemuth, D.J., Celenzo, J., Bundman, D.S. and Dunbar, B.S. (1984) Formation of the rabbit zona pellucida and its relationship to ovarian follicular development. *Developmental Biology*, **106**, 1–14.

Wurtman, R.J. (1964) An effect of luteinizing hormone on the fractional perfusion of the rat ovary. *Endocrinology*, **75**, 927–933.

Yding Anderson, C., Byskov, A.G. and Grinsted, J. (1981) Partial purification of the meiosis inducing substance (MIS) in *Development and Function of Reproductive Organs*, (eds A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 73–80.

Yoshimura, Y., Hosoi, Y., Atlas, S.J., Bongiovanni, A.M., Santulli, R. and Wallach, E.E. (1986) The effect of ovarian steroidogenesis on ovulation and fertilizability in the *in vitro* perfused rabbit ovary. *Biology of Reproduction*, **35**, 943–948.

Yoshimura, Y., Nakamura, Y., Ando, M., Jinno,

M., Oda, T., Karube, M., Koyama, N. and Nanno, T. (1992a) Stimulatory role of cyclic adenosine monophosphate as a mediator of meiotic resumption in rabbit oocytes. *Endocrinology*, **131**, 351–356.

Yoshimura, Y., Nakamura, Y., Oda, T., Ando, M., Ubukata, Y., Karube, M., Koyama, N. and Yamda, H. (1992b) Induction of meiotic maturation of follicle-enclosed oocytes of rabbits by a transient increase followed by an abrupt decrease in cyclic AMP concentration. *Journal of Reproduction and Fertility*, **95**, 803–812.

YoungLai, E.V. and Byskov, A.G. (1983) Relationship of meiotic prophase and ornithine decarboxylase in the neonatal rabbit ovary. *Cell and Tissue Research*, **231**, 565–570.

YoungLai, E.V., Pan, C.C. and Bhavnani, B.R. (1981) Pituitary gonadotropins in fetal and neonatal rabbits. *Biology of the Neonate*, **40**, 199–203.

Zachariae, F. and Jensen, C.E. (1958) Studies on the mechanism of ovulation. Histochemical and physicochemical investigations on genuine follicular fluids. *Acta Endocrinologica*, **27**, 243–355.

Zamboni, L. (1970) Ultrastructure of mammalian oocytes and ova. *Biology of Reproduction*, **2**, Suppl. 2, 44–63.

Zamboni, L. (1972) Fine Morphology of the Follicle Wall and the Follicle Cell–Oocyte Association, *Oogenesis*, (eds J.D. Biggers and A.W. Schuetz), University Park Press, Baltimore, pp. 5–45.

Zamboni, L. (1974) Fine morphology of the follicle wall and follicle cell–oocyte association. *Biology of Reproduction*, **10**, 125–149.

Zamboni, L. (1976) Modulation of follicle cell–oocyte association in sequential stages of mammalian follicle development and maturation in *Ovulation in the Human*, (eds P.G. Crosignani and D.R. Mitchell), Academic Press, New York, pp. 1–30.

Zamboni, I. and Merchant, H. (1973) The fine morphology of mouse primordial germ cells in extragonadal locations. *American Journal of Anatomy*, **137**, 399–435.

Zamboni, I. and Upadhyay, S. (1983) Germ cell differentiation in mouse adrenal glands. *Journal of Experimental Zoology*, **228**, 173–193.

Zamboni, L., Bezard, J. and Mauléon, P. (1979) The role of the mesonephros in the development of the sheep fetal ovary. *Annales de Biologie Animale, Biochimie et Biophysique*, **19**, 1153–1178.

Zeleznik, A.J. (1981) Premature elevation of systemic estradiol reduces serum levels of follicle-stimulating hormone and lengthens the follicular phase of the menstrual cycle in rhesus monkeys. *Endocrinology*, **109**, 352–355.

Zeleznik, A.J. and Kubik, C.J. (1986) Ovarian responses in macaques to pulsatile infusion of follicle-stimulating hormone (FSH) and luteinizing hormone: Increased sensitivity of the maturing follicle to FSH. *Endocrinology*, **119**, 2025–2032.

Zeleznik, A.J., Midgley Jr., A.R. and Reichert Jr., L.E. (1974) Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vivo*. *Endocrinology*, **95**, 818–825.

Zeleznik, A.J., Hutchison, J.S. and Schuler, H.M. (1985) Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides the selection of a single preovulatory follicle. *Endocrinology*, **117**, 991–999.

Zlotkin, T., Farkash, Y. and Orly, J. (1986) Cell-specific expression of immunoreactive cholesterol side chain cleavage cytochrome P-450 during follicular development in the rat ovary. *Endocrinology*, **119**, 2809–2920.

Zoller, L.C. and Weisz, J.C. (1978) The structure and acid phosphatase activity in granulosa cells of preovulatory follicles in rat ovary. *Anatomical Record*, **190**, 592–598.

Zoller, L.C. and Weisz, J. (1979) A quantitative cytochemical study of glucose-6-phosphatase dehydrogenase and [40]D_5-hydroxysteroid dehydrogenase activity in the membrane granulosa of the ovulable type of follicle of the rat. *Histochemistry*, **62**, 125–135.

Zor, U. and Lamprecht, S.A. (1977) Mechanism of prostaglandin action in endocrine glands in *Biochemical Actions of Hormones*, Vol. 4, (ed. G. Litwack), Academic Press, New York, pp. 85–133.

Zuckerman, S. and Baker, T.G. (1977) The development of the ovary and the process of oogenesis in *The Ovary*, 2nd edn, Vol. 1, (eds Z. Zuckerman and B.J. Weir), Academic Press, New York, pp. 41–67.

Zybina, E.V. (1975) An electron microscope study of the lampbrush chromosomes and their activity products during the rabbit's oogenesis. *Tsitologiya*, **17**, 875–880.

PRE-IMPLANTATION DEVELOPMENT 2

C.R. Austin

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2.1 INTRODUCTION

This chapter has interests in common with four chapters in the third edition of *'Marshall'*, and in many instances reference to these is still well justified. The main theme of 'pre-implantation development' ran through Chapter 14 ('Cleavage, early development and implantation of the egg' by J.D. Boyd and W.J. Hamilton, 1952), while pre-implantation stages of parthenogenesis were included in Chapter 10 ('Fertilization' by C.R. Austin and A. Walton, 1960), early anomalies, embryonic losses and delayed implantation (diapause) were considered in Chapter 14 as well as in Chapter 32 ('The endocrinology of pregnancy and foetal life' by R. Deanesly, 1966) and placentation in marsupials was discussed in Chapter 15 ('Placentation' by E.C. Amoroso, 1952). While Chapters 10, 14 and 15 of the third edition contained much information on non-mammalian species, in the present account attention is largely restricted to mammals, in keeping with the title of this volume, brief mention of features in non-mammals being made when perspective may benefit thereby.

It is fitting next to clarify the use of certain terms. 'Egg' has commonly been employed in the past for the developing entity at stages up to and including implantation, and if the same general size and form are retained, as in man and several other mammals, the label is appropriate. But it is less apt after implantation, or even before implantation in ungulates, because of major changes in morphology, and it carries quite a different connotation when applied to the much larger and more nutrient-laden egg of the monotreme. 'Ovum' has similar virtues and faults; in addition, in the medical literature, the word can denote the entire implanted structure, or even be employed in abnormal states when only membranes persist, as in 'amniotic ovum', an amniotic sac expelled after abortion and resembling a complete conceptus; 'blighted ovum', in which there is a chorion but no embryo; and 'dropsical ovum', a hydramniotic conception sac also lacking an embryo. 'Conceptus' has wide currency and a general connotation, for it is appropriate throughout pregnancy; by contrast, 'zygote' (the fusion product of gametes: Gk: *zygon*, a yoke) is a bit ambiguous, for though it seems properly restricted in its use to describing the fertilized egg during the period between syngamy and cleavage, a very brief phase in mammalian development, many authors grant it an indefinitely longer life.

The most popular label is 'embryo' (Gk: signifying 'something growing within'), but here too there are difficulties, and these will be discussed in some detail for it is important that our terms should be as unambiguous as possible. In human medicine, 'embryo' is commonly used from the time of fertilization to about the eighth week of pregnancy, by which time resemblance to the human form is undeniable; thenceforth until birth, the little creature is a 'fetus'. In the study of other mammalian species, the term embryo has been employed up to the time of birth (or hatching, in monotremes). Both usages are open to objection for they imply the occurrence of no major change, other than increasing size and complexity – a notion apparently deriving from invertebrate embryology. But in mammals the fertilized egg and its pre-implantation products are not equivalent to the fetus or the future individual – much more than that, they also give rise to the placenta and fetal membranes, which in the early stages possess far greater mass and detail.

We should also ponder the following relevant points. After fertilization, the succession of cell divisions or 'cleavage' begins, whereby a ball of cells, the 'morula', is formed, in which all cells have full developmental potential and are therefore said to be 'totipotent'. With further cell division and the formation of a cavity, the morula becomes a

'blastocyst'; in this there are two cell types, making up the 'inner cell mass' and the 'trophectoderm'. The more numerous trophectoderm cells early undergo some differentiation and become irrevocably committed to the formation of placenta and fetal membranes, and they cannot then function as contributors to the fetus itself. By contrast, the cells of the inner cell mass are initially uncommitted and so described as 'pluripotent'. As development proceeds, the inner cell mass differentiates into 'primitive ectoderm' and 'primitive endoderm' (see Table 2.1) and among the subsequent products of the former a small group of cells becomes distinguishable that alone is destined to constitute the fetus. It is this select little tribe that properly merits the term 'embryo'. This distinction has important developmental and functional significance.

In work involving human pre-implantation development, there may well be ethical and legal reasons for pursuing the matter of definition further, for the embryo, at the stage just described, could be held equivalent to the future 'person'. However, some developmental flexibility yet remains. In the human subject, the group of cells constituting the embryo becomes clearly identifiable during the second week after fertilization as the 'embryonic disc', and this is capable of dividing into two or more discs, thus giving rise to twins, triplets, quadruplets, etc. Only after that phase is over do embryos have strict individuality. The term 'embryo' as thus defined creates a need for appropriate labelling of the entire complex of cells that existed prior to the appearance of the embryonic disc, and 'proembryo' would appear to be a sensible choice. (The proposed use of this term here, already well established in the botanical literature, is consistent with its definition as given in *Encylcopeadia Britannica* (including *Macropaedia* and *Micropaedia*), *Henderson's Dictionary of Biological Terms*, *Chambers Science and Technology Dictionary* and other works of reference. Some writers use the spelling 'pre-embryo', but 'proembryo' is etymologically preferable.)

The range of development that can be described as 'pre-implantation' varies with the species. In man and several other mammals, attachment and implantation (nidation) occur relatively early and involve the unilaminar unexpanded blastocyst. At the other extreme, the blastocyst in ungulates undergoes enormous expansion together with the formation of additional structures, and it is indeed an 'embryo' (no longer a proembryo), with chorion, yolk sac and allantois, when it eventually forms attachment to the endometrium. Finally, in monotremes and some marsupials, integration of embryonic membranes with the endometrium never occurs, so that the terms 'attachment' and 'implantation' have no relevance; for these animals, it seems most reasonable in this chapter to follow development to the point at which birth or hatching takes place.

In this introduction, the contributions made by earlier workers to our understanding of the pre-implantation stages of development merit at least brief mention. One of the first to accept the notion of the ovary as the source of eggs, in mammals as in birds, was de Graaf (1672), and he looked for and found eggs in the oviduct and uterus, but thinking that the ovarian follicle was the egg at source was bewildered at the difference in size. Nobody could confirm his findings in the oviduct and uterus or was willing to support his concept until over 100 years later, when Cruickshank (1797) reported that he had recovered eggs from the rabbit oviduct, from 3 days after coitus onwards, and also from the uterus, but he could not trace eggs back to their origin. So it fell to von Baer (1827, 1837) to be the first to identify the ovarian egg (in the dog and sheep), and then Barry (1838, 1839) provided some very clear illustrations of two- and four-cell eggs and morulae of the rabbit. Shortly afterwards, Bischoff (1842a,b, 1845, 1852, 1854) described many cleavage stages with even greater precision in the rab-

bit, dog, guinea pig and deer. In this way, the initial stages of mammalian development came to be appreciated, but purely in a pictorial sense, for the true nature of eggs and blastomeres as specialized cells was not understood, and the details and significance of cell division and fertilization had yet to be elucidated.

The beginnings of a deeper understanding came with the foundation of the 'cell theory', a component of which was Brown's recognition in 1831 of the nucleus as an essential element in the cell, and the principle of which was first clearly stated in 1838 and 1839 by Schleiden and Schwann (as helpfully explained by Nordenskiöld, 1928, and Wilson, 1928). Further elaboration and refinement was made by several other authors during the succeeding half-century, and this, together with the demonstration by Gegenbaur in 1861 that the egg is a single cell, led to the realization that all tissues and organs – and organisms – were composed of cells, and that cells always arose from pre-existing cells, generation after generation, as a continuum reaching back to the primordial forms of life. At last, cleavage began to have meaning as the initial assemblage of building blocks for the ordered construction of the new individual.

2.2 THE CLEAVING EUTHERIAN EGG AND THE BLASTOCYST

2.2.1 CLEAVAGE AND TRANSPORT PATTERNS AND RATES

Cleavage consists in successive nuclear and cytoplasmic divisions of the fertilized or otherwise activated egg, the products being held together initially by the encircling zona pellucida. Cleavage, following fertilization of the eutherian egg, resembles superficially that of certain marine invertebrate eggs which have been studied in great detail (see Wilson, 1928). In coelenterates and sponges, cleavage is random and unrelated to the distribution of cytoplasmic components, but in most other invertebrate groups it proceeds in an orderly manner according to a clear-cut pattern, so that the egg components are segregated in conformity with the nature of the cells and tissues that will develop in the embryo and later in the adult organism. These random or ordered systems are distinguished by the terms 'indeterminate' and 'determinate' cleavage respectively. Eutherian eggs conform rather to the former system, at least during the first few cleavage divisions (Figure 2.1, and see Figures 14.9–14.16 in the third edition of *Marshall*); subsequently, order and pattern become distinguishable, and the cleavage form can then be classed as determinate. The later segregation of cell types in mammalian development is unique in that among miolecithal eggs it is only those of mammals that are called upon to produce placenta and extraembryonic membranes in addition to the future organism (Table 2.1).

Rates of cleavage are moderately consistent among the eutherian species that have been studied, the first division occurring about 24 h after ovulation – that is, with intercurrent fertilization, for parthenogenetic cleavage is initiated a little later.

Subsequent divisions take place at about 12-h intervals; this is an average figure, for divisions in any one egg are not exactly synchronous and eggs with odd numbers of blastomeres are not uncommon. There is some evidence that ungulate eggs cleave more slowly than those of other eutherians, but published figures show much variation. In the mouse, it has been shown that the rate of cleavage is to some extent genetically determined (Whitten and Dagg, 1961). In polytocous animals, eggs in the same individual often differ by one or more divisions, the asynchrony increasing as cleavage proceeds. (Data on cleavage rates have been tabulated by Boyd and Hamilton, 1952; Blandau, 1961; Brinster, 1972; and McLaren, 1982.) Tsunoda *et al.* (1985a) have reported finding that fast-cleaving mouse eggs gave rise after transfer

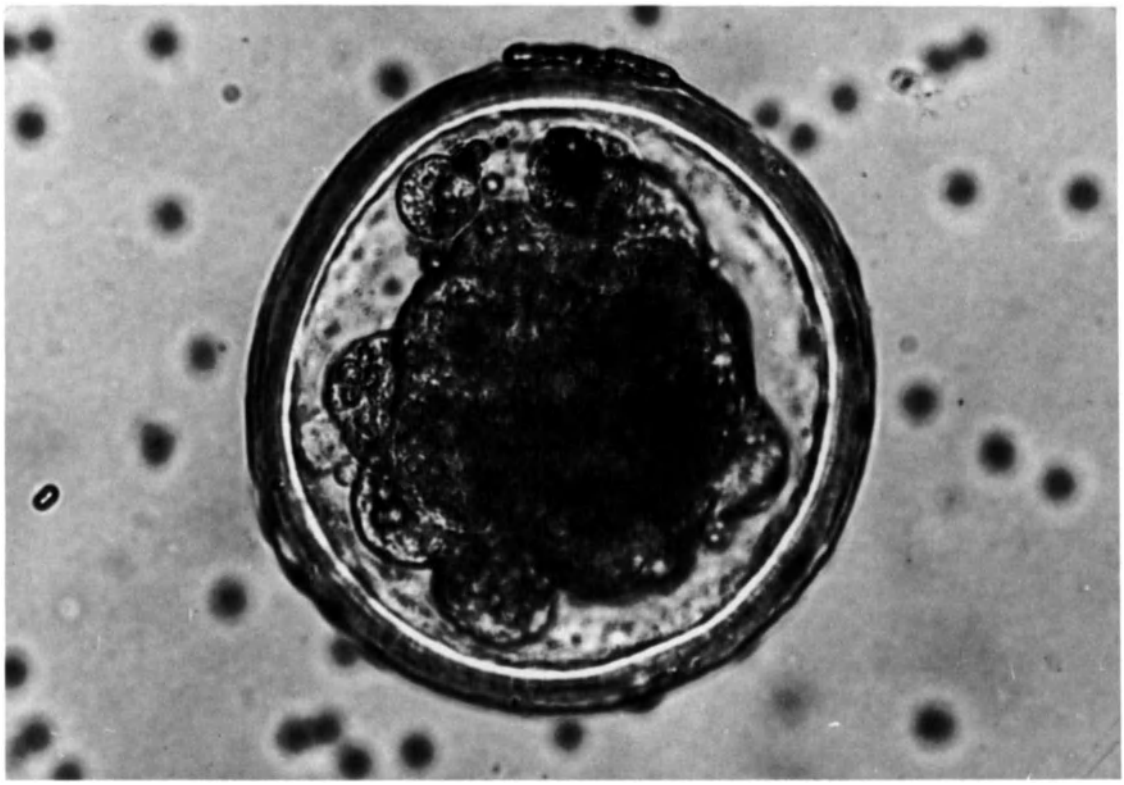

Figure 2.1 Sixteen-cell egg from a swamp buffalo *Bubalus bubalis*, 150μm in diameter. (Micrograph by courtesy of Professor Maneewan Kamonpatana, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.)

into recipients to 71% male mice, whereas the slow-cleaving eggs yielded 80% female mice.

Cleavage of the fertilized or parthenogenetically activated egg continues until cell size and nucleocytoplasmic ratio are similar to those of the average tissue cell. With each division, the cytoplasmic volume of blastomeres is approximately halved. The total cytoplasmic volume does not remain constant but slowly and progressively diminishes, presumably because yolk materials are used up to yield components necessary for maintenance and in division; in addition, as explained later, energy resources are taken up from the surrounding medium. The reduction in cell volume from the one-cell stage to the eight-cell stage has been found to be about 20% in the cow, 40% in the sheep, 30% in the ferret and 25% in the mouse (Hamilton and Laing, 1946). The nature of the cue for each cleavage division is not understood; with tissue cells which grow in size during interphase, the cue is evidently the acquisition by the cell of a critical volume, but in cleavage the cells become progressively smaller. Because divisions are not exactly equal, the blastomeres in the morula tend to be graded in size and they aggregate accordingly; the gradation was thought to portend different fates (Boyd and Hamilton, 1952), especially if associated with differences in cytoplasmic features, including staining

Table 2.1 Cell lineages during pre-implantation and early post-implantation development, as determined for the mouse (see Gardner, 1982). Numbers in parenthesis represent the average numbers of cells recorded. Italicized items are those that will constitute the embryo (as distinct from proembryo – a point discussed in the Introduction).

Days post coitus				
	Morula			
3.5	Trophectoderm (TE) (45)		Inner cell mass (15)	
4.5	Mural TE	Polar TE	Primitive ectoderm	Primitive endoderm
	(50)	(15)	(22)	(25)
> 5	Primary trophoblastic giant cells	Ectoplacental cone and extra-embryonic ectoderm	Amniotic ectoderm Extraembryonic mesoderm *Ecto-, meso- and endoderms of fetus* *Germ cells*	Visceral and parietal endoderms

properties (Dalcq, 1957). Current views on cell fates in early development are discussed later in section 2.2.4.

Fertilization in most mammals takes place in the ampulla of the oviduct, being completed within about 24 h of ovulation. As the egg undergoes cleavage, it is moved along the oviduct and into the uterus – in 2–3 days in most instances, but some 5-6 days in the dog, cat and ferret, and 8 days in the mink. (Times of entry into the uterus in various animals have been tabulated by Boyd and Hamilton 1952; Blandau, 1961; Brinster, 1972; and McLaren, 1982.) Transport is attributed to contractions of muscle layers in the wall of the oviduct, and the activity of these muscle cells is readily influenced by certain hormones and drugs (see Coutinho, 1974). Ciliary action may also play a part in transport of the cleaving egg (the evidence for such a function is much more convincing during transfer of the just ovulated egg to the ampulla of the oviduct – Blandau, 1973). Mysteriously, while fertilized eggs in the horse are transported into the uterus, unfertilized eggs tend to be left behind in the oviduct (Betteridge and Mitchell, 1974). Apparently uniquely, in man normal transport may occasionally fail so that the egg becomes implanted in the oviduct, or, being lost from the oviduct through retrograde transport, passes into the peritoneal cavity and implants there; under both circumstances, the result can be an 'ectopic' pregnancy. (For a WHO report on factors considered conducive to ectopic pregnancy, see Anon., 1985.) In animals lacking a complete ovarian capsule, it is possible for eggs to pass across the peritoneal cavity from the ovary on one side to the contralateral oviduct, be fertilized there and implant in the corresponding uterine horn. This adventure is termed 'external migration'; its occurrence posited by some and denied by others, an experimental enquiry yielded negative results (Parkes, 1924). Oviduct function is well

reviewed in a text edited by Hafez and Blandau (1969).

2.2.2 DISTRIBUTION IN UTERUS

Blastocysts in polyoestrous animals may be moved around a great deal after entering the uterus and before implantation or attachment, a period that ranges from a day or two in the mouse and rat to 24–30 days in the horse and more than 30 days in the cow. Much longer periods are, of course, met with in animals that exhibit diapause. The movement is effected by muscular activity in the wall of the uterus, which can be demonstrated to distribute other objects as well, such as sea urchin eggs, small pieces of muscle tissue and plastic beads. Where there is free communication between the two horns of the uterus, as in the horse, sheep, pig, goat and dog, blastocysts deriving from the ovary on one side often become implanted or attached in the contralateral uterine horn (as observed in the dog by Bischoff, 1845). Movement of this kind is sometimes called 'internal migration' (Boyd and Hamilton, 1952, gave a list of 18 species in which it has been recorded, including five species of bats that exhibit implantation nearly always in the right horn, although ovulation occurs in either ovary). It would be most unlikely to take place in animals like the rat, in which the lumina of the cervical canals remain separate for their full extent; it is rare in the mouse, in which the cervical canals anastomose only a short distance from the external os. Internal migration was early shown to be a common occurrence in the sheep by Boyd *et al.* (1944); by contrast, it is rare in the cow.

2.2.3 CYTOLOGY OF CLEAVAGE AND COMPACTION

Fundamental to the processes of fertilization, and for cleavage and compaction (this section) and the fusion of oocytes for the initiation of development (discussed later under Induction of parthenogenesis, section 2.4.4), are the properties of the plasma membrane, which have been the subject of a review by Wolf (1983).

Following the aggregation of chromosome groups at syngamy, which can be considered the concluding step of fertilization, a series of mitotic cell divisions occurs, constituting the process of cleavage. During each interphase, DNA replication takes place in the 'S' phase, preceded and followed by 'gap' phases (G_1 and G_2). During G_1, RNA and protein are synthesized in preparation for DNA replication, and during G_2 the same syntheses occur for cell division. G_1 is lacking in the first few cleavage divisions (see Grant, 1978).

In the eight-cell mouse egg, a distinctive change is soon evident, marked by closer and more extensive apposition between blastomeres (Figure 2.2).

The cells become wedge-shaped rather than oval, and are now polarized so that their inner and outer regions exhibit different characteristics. The outline of the cell mass becomes more even and barely suggests the existence of separate cells. This change is called 'compaction'; it has been recognized in all species investigated for its occurrence and is evidently an essential step towards the next new feature, the development of a fluid-filled cavity among the blastomeres, leading to the formation of the blastocyst (the series of events is well illustrated in Figure 2.3, from Kaufman, 1983; ultrastructural changes during the corresponding stages of development in the mouse egg have been described by Cech and Sedlackova, 1983).

Maintenance of the fluid-filled cavity depends on the presence of zonular tight junctions between contiguous cells and the ultrastructure of these components can be seen to develop during the life of the eight-cell egg (Ducibella and Anderson, 1975; Ducibella *et al.*, 1975; Ducibella, 1977) (Figure 2.2). Such tight junctions prevent the escape of fluid and limit the movement of ions, thus maintaining a distinctive environment in the

cavity of the blastocyst (the blastocoele). 'Gap junctions' are also present, having the inferred function of enabling communication between cells by the passage of electrolytes and other solutes. (The nature and significance of compaction are discussed by Johnson *et al.*, 1981.)

2.2.4 THE BLASTOCYST

The eutherian blastocyst resembles the blastula of many marine invertebrate animals in a general way, but differs in the fact that the entire blastula goes to form the new individual, whereas only a small proportion of cells of the blastocyst subserves this function. (The distinction between the two terms is not universally recognized but will be observed in this chapter.)

For early observers of eutherian eggs in late cleavage it was tempting to interpret differentiation along similar lines to those previously demonstrated for invertebrate eggs of the 'mosaic' variety, and there were claims that appropriately disposed cytoplasmic determinants existed, distinguishing the cells destined to constitute the outermost (enclosing) layer, now identified as the 'trophectoderm', from the enclosed group of cells which form the 'inner cell mass' (ICM) (Dalcq, 1957; Mulnard, 1965). But later investigators, including Kelly (1975, 1979), were not able to support these claims, finding that individual blastomeres of the eight-cell egg could contribute to either trophectoderm or ICM.

The basis for present-day thought on the subject was established by Tarkowski and Wroblewska (1967), who proposed that the blastomeres of the early cleavage stages were intrinsically capable of differentiating as trophectoderm cells, but could gain the capacity to become ICM cells through residence in an intercellular environment – this was their 'inside-outside hypothesis'. The formation of a distinctive intercellular environment follows from the establishment of the system of tight junctions, which distinguishes the regions of contact between trophectoderm cells and which enables blastocyst expansion to occur; the tight junctions become recognizable when compaction occurs.

An alternative mechanism has been proposed by Johnson and his colleagues (Johnson, 1981; Johnson *et al.*, 1981), and this involves two processes: the 'conditioning' of cells by contact with others for further contacts (the progeny of the first cell of the two-cell egg to divide will have more contacts and

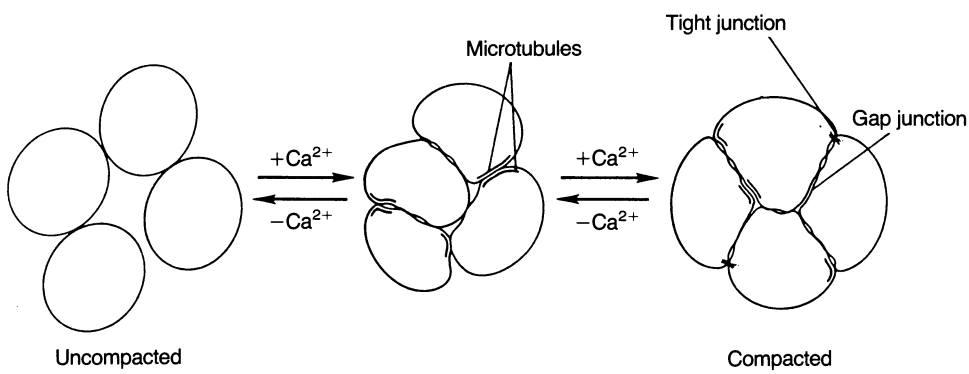

Figure 2.2 Diagram illustrating the process of compaction as seen occurring between four cells of an eight-cell egg. Changes involve the close approximation of cell surfaces and the development of tight junctions, which will later make possible the accumulation of blastocyst fluid. Surface and junctional changes depend on the presence of calcium ions in the medium, and compaction is reversed if the egg is placed in a calcium-free medium. (After Ducibella, 1977.)

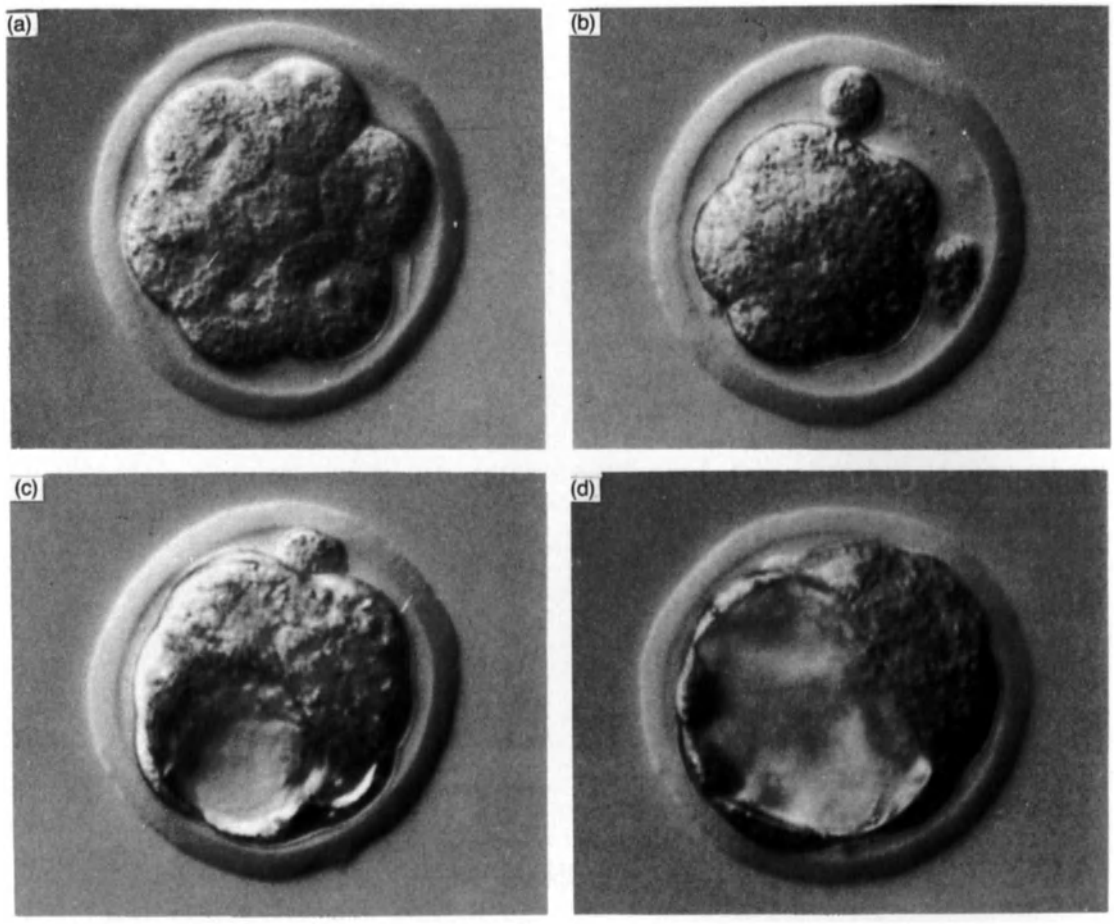

Figure 2.3 Compaction and blastocyst development as seen by interference microscopy in living mouse eggs. (From Figure 6.2 in Kaufman, 1983.)

so become more amenable to contact) and the 'polarization' of blastomeres at the eight-cell stage, as a result of which each cell comes to possess a limited region of 'outer' surface characterized by the presence of microvilli. Division of blastomeres at the eight-cell stage can either bisect the microvillus area (creating two potential trophectoderm cells) or segregate it into one daughter cell; daughter cells without this surface area become internalized and later form the ICM. Commitment to an ICM fate may not be final, however, for there is evidence that cells in the early ICM may come to take part in the trophectoderm (Rossant, 1986).

For further discussion of these subjects see Adamson and Gardner (1979), Johnson *et al.* (1984), Nichols and Gardner (1984), Johnson (1985) and Mulnard (1986).

2.2.5 SPECIES DIFFERENCES IN BLASTOCYST DEVELOPMENT

In animals whose blastocysts do not exhibit an inner cell mass, but instead a group of cells specialized for functions equivalent to those of the inner cell mass though taking up a position alongside cells constituting the trophectoderm, as in the pig (Heuser and Streeter, 1929), the goat (Amoroso *et al.*, 1942)

(Figure 2.4) and the sheep (Bryden *et al.*, 1972), and in marsupials (see Chapter 7), it is still uncertain how the distinction between the two groups of cells arises. The cleavage stage at which blastocyst formation begins varies (Figure 2.5).

In mouse, rat and man, the first evidence of the blastocoele (Gk: *koilos*, hollow) appears shortly after compaction, when there are between 16 and 32 cells present; in the rabbit, the corresponding cell number is around 100–200. Experiments with rodent and rabbit eggs, however, have shown that the time of cavitation is not directly dependent on the number of blastomeres that have been formed. If blastomeres are removed from mouse early cleavage stages, even as many as three from the four-cell egg, intercellular fluid will still accumulate at the usual time, producing in this instance a 'blastocyst' with only three or four cells – sometimes, miraculously, when only two cells are allowed to remain they may contrive to embrace a blastocoele between them. Available evidence indicates that the time of fluid accumulation is linked to the number of mitoses that have occurred, and not to the number of cell divisions (which can be independently suppressed).

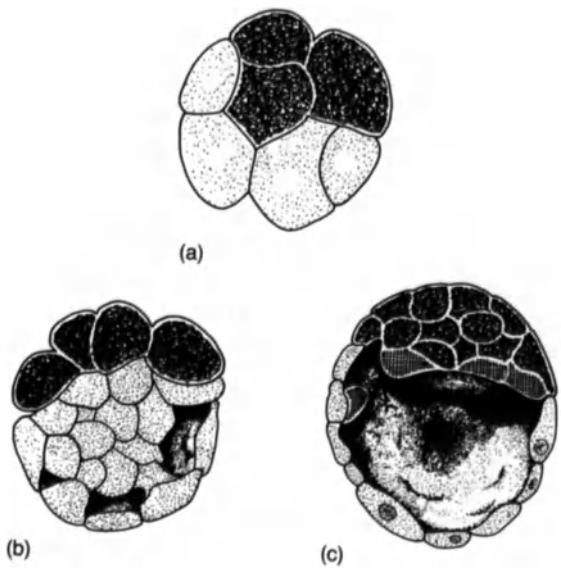

Figure 2.4 Blastocyst formation in the goat egg is characterized by the lack of development of an inner cell mass. The future embryo derives from some of the surface cells, distinguished here by white stipple on a black background. Cross-hatched cells are those of the developing endoderm. (After Amoroso *et al.*, 1942).

In mouse, rat, hamster, guinea pig, some bats, monkey and man, formation of the blastocyst involves increase in size of the blastocoele and in the volume of blastocoelic fluid, with little if any increase in the overall diameter, accommodation being provided by decrease in cytoplasmic mass of the cells and virtual disappearance of the perivitelline space. Some further expansion occurs when the blastocyst escapes ('hatches') from the zona pellucida, but at implantation the diameter of the blastocyst is only about 50% greater than that of the morula. Hatching is made possible through erosion of the zona by lytic action of the uterine secretions, a property that appears to be under hormonal control for it exists only at about the time that implantation normally occurs. Some lytic action is also exerted by cells of the blastocyst, especially those developing the pseudopodia-like protrusions that extend from the pole opposite to that occupied by the inner cell mass, as clearly demonstrated in the guinea pig by Blandau (1949).

In some bats, insectivores, and the cat and dog, some expansion of the blastocyst occurs, while in the rabbit and hare the diameter increases much more, reaching 3–4 mm, compared with about 90 μm for the morula without zona or mucin layer. Expansion of the rabbit blastocyst has drawn a good deal of research attention because of the evidence that this event depends on the presence of a specific protein in uterine secretions, which become especially abundant as the time of implantation approaches (named 'uteroglobin' by Beier, 1968, and 'blastokinin' by Krishnan and Daniel, 1967, 1968). Recently, however, expansion has been reported to

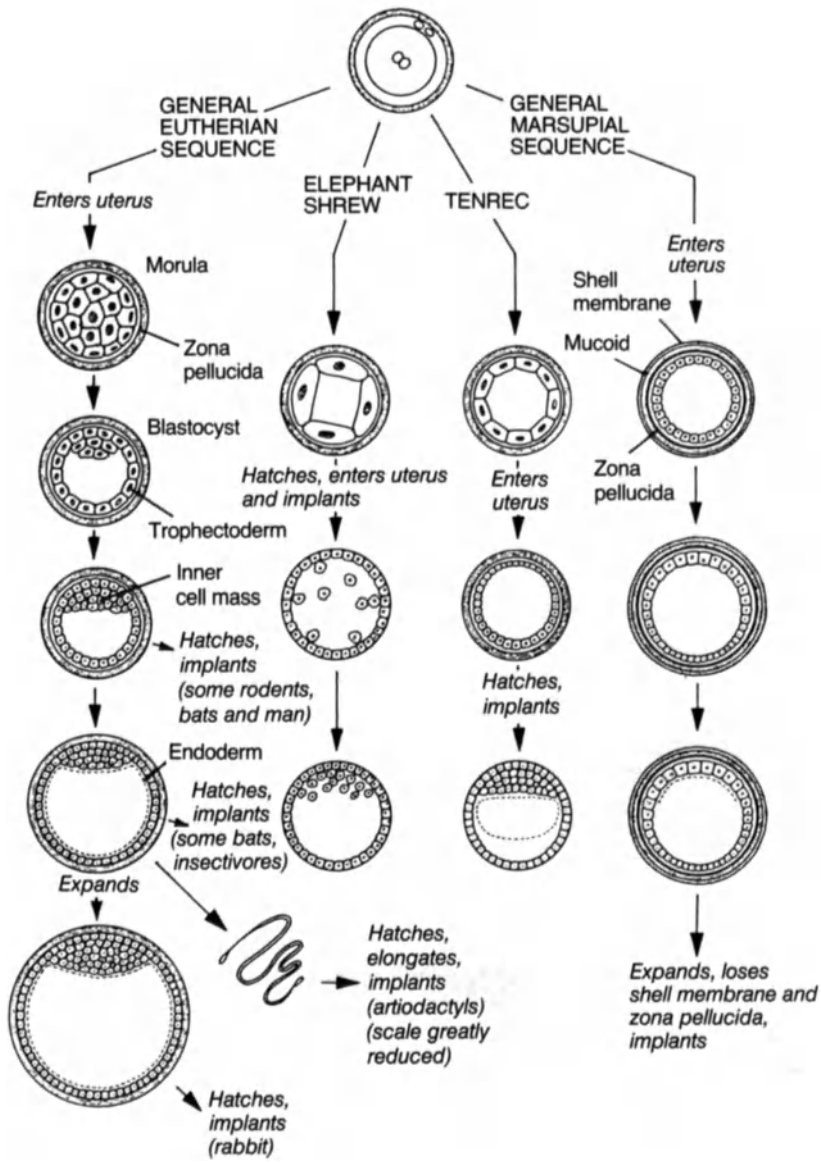

Figure 2.5 Stages in the development of the blastocyst in different mammalian groups. (From McLaren, 1982, after Wimsatt, 1975.)

occur in a simple synthetic medium (see McLaren, 1982). In the pig, a uterine-specific protein has been described with a purple fluorescence (and therefore called 'purple protein'); its synthesis is stimulated by progesterone and its function is thought possibly to be promotion of placental development (Murray *et al.*, 1972).

In the horse and its relatives, the blastocyst expands greatly, achieving a large, more or less spherical structure, before implanting on day 28 after ovulation. Expansion in the

sheep, cow, goat, pig, deer, etc. is also considerable, but the distinctive feature here is the extreme elongation and attenuation that the blastocyst undergoes, assuming a tubular form which can reach a length of a metre and a half or more in the pig (Perry and Rowlands, 1962). Implantation occurs relatively late, from some 15 days after ovulation in the pig up to 35 days in the cow. Development, however, proceeds apace during the long pre-implantation period, and reaches a much more advanced stage than in animals with early-implanting blastocysts.

It is at the blastocyst stage that the first clear evidence of differentiation appears, with the presence of two types of cell, but their disposition varies among mammals. In a number of species, such as the laboratory rodents, rabbit and man, the 'outside' cells of the late morula predominantly differentiate into the trophectoderm (Gk: *trophe*, nourishment, *ekto*, outside, *derma*, skin), which makes up the enclosing wall of the blastocyst, while the 'inside' cells remain undifferentiated and form the inner cell mass, a distinctive group at one pole. Shortly before implantation, the inner cell mass differentiates into primitive ectoderm and primitive endoderm; from some of the cells of the former the embryo later develops (Table 2.1). In ungulates, such as the sheep and goat, the initial differentiation appears to be directly into trophectoderm and primitive ectoderm; the latter makes a group of cells that projects into the blastocoele but also forms part of the outer wall, the remainder of the wall being trophectoderm. The primitive ectoderm is the homologue of the inner cell mass; that term is clearly inappropriate here. Other variations in the process of blastocyst formation also exist among eutherian mammals (see Wimsatt, 1975) (Figure 2.5).

In animals with a long pre-implantation or preattachment period, a fourth type of cell becomes identifiable, forming the mesoderm. The blastocyst has thus advanced from its initial state of unilaminar through bilaminar to trilaminar, the layers soon coming to constitute the embryonic membranes 'chorion', 'amnion', 'yolk sac' and 'allantois'. The remaining cells of the primitive ectoderm become organized as a flat oval or pear-shaped body known as the 'embryonic disc', soon to be embellished with the 'primitive streak', consisting of two folds with a groove in between. In the sheep, the embryonic disc is evident at 13 days after ovulation, being about 0.42 mm in length. 'Neural folds' appear on either side of the primitive streak by the 15th day, seven somites then develop and the embryo reaches about 1.60 mm in length; during this period, there is a rapid decline in glucose incorporation by the developing tissues (Cuneo, 1986). The neural groove closes on the 16th day and the 'heart bulge' is discernible by the 17th (Bryden *et al.*, 1972). By day 19, the embryo has reached 7.76 mm in length and shows a 60-fold increase in dry weight as compared with day 13 (Cuneo, 1986). The first evidence of a reaction in the endometrium to the presence of the embryo is seen about days 15–16 (Boshier, 1969) and the first responsive changes in the trophoblast cells at days 13–15 (Guillomot *et al.*, 1982).

2.2.6 X-INACTIVATION

In most eutherian mammals, the compensating mechanism avoiding the aneuploidy otherwise due to the presence of two X chromosomes in female tissues involves inactivation of one of them (Lyon, 1961). Studies on the development of sex chromatin (or Barr body) in man, macaque, cat and rabbit indicated that inactivation occurs at the blastocyst stage; sex chromatin becomes visible first in the trophectoderm, and later in the derivatives of this tissue, namely the primitive ectoderm and primitive endoderm. (In eutherian mammals, inactivation can be of either the paternal or maternal X chromosome, and in a random manner, except in the

extraembryonic membranes where inactivation is preferentially of the paternal X. In marsupials, it is the paternal X chromosome that is inactivated in both tissues.) Replication asynchrony of X chromosomes is also detected first at the blastocyst stage, in conformity with the observations on sex chromatin.

On the other hand, there is good evidence from the X-linked gene dosage effect on enzyme synthesis that both X chromosomes in female embryos are transcriptionally active at the eight-cell stage and probably earlier. No evidence of dosage compensation has been detected, so it is inferred that only a very small part of the genome is active at this time. Dosage studies have also shown that X-inactivation at the genetic level must begin earlier than that indicated by the appearance of sex chromatin, perhaps as early as the morula stage, and then progressively increases.

The first appearance of sex chromatin in the trophectoderm may have a further point of significance, for there is accumulating evidence that X-inactivation occurs as cells differentiate, supporting observations on teratocarcinoma cells that were induced to differentiate (see Lyon, 1972; Gartler and Cole, 1981; Monk and Harper, 1979, for further details on these and preceding observations).

2.2.7 VIRUS-LIKE PARTICLES

There have been numerous reports on the presence in cleaving eggs of small round objects, 50–110 nm in diameter, that resemble RNA tumour viruses. They have electron-dense shells and commonly look as if they were budding off the outer layer of the nuclear envelope or from the endoplasmic reticulum, but often, too, they are found free in the cytoplasm. They appear to exist in three different forms, which have been labelled types A, B and C (Dalton, 1972). The particular form exhibited appears to depend on the developmental state of the egg. The particles have been found in early germ cells, in gonocytes and oogonia, and in immature oocytes up to the dictyate stage (Larsson *et al.*, 1981), but never in unfertilized one-cell eggs. After fertilization, they are first seen in the late two-cell egg and then in all stages up to the egg cylinder. Calarco and Szollosi (1973) noted the apparent coincidence between the presence of the particles after fertilization and the beginning of RNA synthesis by nucleoli. They have also been seen in fetal tissues. Sometimes crystalloid aggregates are also seen in eggs that contain virus-like particles, particularly at the two-cell stage (Szollosi, 1976). Dvorak *et al.* (1985) did not record virus-like particles, but found crystalloid inclusions to increase in abundance from the four-cell stage onwards; fibrillar proteins were found to be components of these inclusions.

Most of the observations mentioned so far were made in the mouse, but there have been scattered sightings in the guinea pig, hamster and some other animals. Virus-like particles have also been noted in parthenogenetically activated mouse eggs, first at the two-cell stage and in smaller numbers up to the morula (Van Blerkom and Runner, 1976). Daniel and Chilton (1978), who present a comprehensive review of this field, propose that virus-like particles could represent messengers for cell-to-cell communication during embryonic development.

More recently, Yotsuyanagi and Szollosi (1980, 1981) have described what appears to be a new type of inclusion, termed 'epsilon particle', found in mouse eggs from the two-cell stage to the morula. In the earlier eggs, the particles seemed not to exert any ill-effect, but the presence of large numbers in the morula appeared to be associated with loss of contact between an affected blastomere and neighbouring cells, which might lead to its exclusion from the blastocyst.

2.2.8 DIAPAUSE

This term denotes an arrest of development occurring as a normal but not invariable event in early pregnancy; it is also known as 'delayed implantation' and 'discontinuous development'. The qualifications 'facultative' and 'obligatory' are often met with, but the corresponding terms 'seasonal' and 'lactational' are preferred because the imputations are clearer. The literature on diapause was thoroughly reviewed by Deanesly (1966) in the third edition of *Marshall*; she listed 18 eutherian species on which information had been published (see also the reviews by Aitken, 1977; Bergstrom, 1978; Renfree, 1978; Flint *et al.*, 1981).

Seasonal diapause became known first in the roe deer *Capreolus capreolus* and the nine-banded armadillo *Dasypus novemcinctus*. In the roe deer, fertilization, implantation and birth usually occur in August, December and May, in that order; for the armadillo, the relevant months are July, November and March–April. For both animals, the time between the first two events represents more than 40% of the whole pregnancy period. Even more striking is the European badger *Meles meles*, in which the three events occur in July, February and March, the pre-implantation interval here approaching 90% of pregnancy. In these species, delay of implantation ensures that birth of young occurs in a favourable season of the year, and so is appropriately termed 'seasonal diapause'. The stimulus for this reaction evidently has to do with the photoperiod since the artificial provision of 'increasing day length' has been found to induce premature implantation. Temperature and nutrition are also involved.

Lactational diapause has long been known in rats and mice, which can mate at the first post-partum oestrus and would thus be suckling a litter when the blastocyst enters the uterus. It involves an additional interval of up to 20 days in the blastocyst's intrauterine existence, the actual duration being proportional to the number of young being suckled. Usually at least five young in the rat and three in the mouse are necessary for the response. Studies in the mouse have shown that the metabolic rate of diapaused blastocysts, as indicated by the rate of glycolysis, is much reduced. Lactational diapause has also been reported in the shrew *Sorex araneus*.

The mechanism in rodents involves a balance between oestrogen and progesterone. If very small amounts of oestrogen are injected into mated lactating mice and rats on days 5–6, implantation occurs normally, while delay ensues in the controls, but if the animals are ovariectomized and maintained on progesterone, the blastocysts remain unimplanted (see McLaren, 1973; Weitlauf, 1974). In mice, blastocysts can be induced to enter diapause by transfer to the oviducts of immature mice; reactivation follows removal to pseudopregnant mice or culture *in vitro* (Papaioannou, 1986). In the rabbit, hamster, guinea pig, sheep and rhesus monkey, diapause induced by ovariectomy can be terminated and implantation follows if progesterone alone is administered, oestrogen apparently being unnecessary (Renfree, 1982). Naaktgeboren *et al.* (1986) have recently described eight cases of induced human pregnancies in which there was evidence of delayed implantation; two pregnancies went to term with healthy births.

2.2.9 METABOLISM

Some of the earliest observations on the metabolism of the pre-implantation stages of development were concerned with respiratory activity and were made by Boell and Nicholas (1948) in the rat and A.H. Smith and Kleiber (1950) in the rabbit, and these were followed by the investigations of Fridhandler *et al.* (1957) and Fridhandler (1961) in the rabbit and Sugawara and Umezu (1961) in the rat. Oxygen uptake was measured by the Cartesian diver technique. It was noted in the rabbit that later pre-implantation stages

had higher oxygen consumption rates, and that the earlier stages were evidently not dependent on the presence of substrate metabolites, though these stimulated oxygen uptake in the more advanced stages. Subsequent research revealed an increasing metabolic capability in the pre-implantation stages of the mouse: in the oocyte and ootid, only pyruvate and oxaloacetate could be metabolized in significant amounts; at the two-cell stage, phosphoenolpyruvate and lactate could additionally be utilized, and at the eight-cell stage the list included also glucose, α-ketoglutarate, malate and acetate (Table 2.2).

Despite these differences, all stages from two-cell to eight-cell were found to be equally permeable to glucose. In the eight-cell and morula stages, about half the glucose metabolized enters pathways leading to the synthesis of proteins, lipids and nucleic acids. (For references see Biggers *et al.*, 1967; Biggers and Borland, 1976; Brinster, 1967a,b, 1972; Wales and Brinster, 1968; Wales, 1973, 1986; Sherman, 1979.)

The oxygen consumption of human oocytes and blastocysts, the latter arising from *in vitro* fertilization (IVF), was found to be essentially similar (ranging from 0.34 to 0.53 nl/h) by Magnusson *et al.* (1986), and Leese *et al.* (1986) reported that the pyruvate uptake by human oocytes decreased after fertilization but rose again just before the morula stage, the changes being considered of possible value for assessing viability. Gott *et al.* (1990) found that more pyruvate than glucose was taken up by human IVF embryos in the earlier stages, but glucose was the preferred substrate for the blastocyst. The production of lactate was relatively high throughout, values observed beginning at 43.6 pmol/embryo/h on day 2.5 and rising to 95.4 pmol/embryo/h by day 5.5. The authors consider that only about half the lactate produced could have come from the glucose taken up, the rest deriving most likely from glycogen.

The metabolism of glucose and lactate in pre-implantation mouse embryos was increased by 62% and 18%, respectively, by culture in the presence of platelet-activating factor (PAF), as reported by Ryan *et al.* (1990a,b), who suggest that embryo-derived PAF has a role in pre-implantation development, augmenting the viability of the embryos themselves.

Important reserves of energy for the cleaving egg are the glycogen stores observed in mouse eggs by Thomson and Brinster (1966) and Stern and Biggers (1968). The distribution of glycogen granules and aggregates at different developmental stages has been

Table 2.2 Metabolites that can provide energy for the oocyte, ootid and cleaving mouse egg (From Brinster, 1972)

	Oocyte	*Ootid*	*Two cells*	*Eight cells*
Pyruvate	+	+	+	+
Oxaloacetate	+	+	+	+
Lactate	−	−	+	+
Phosphoenolpyruvate	−	−	+	+
Glucose	−	−	−	+
α-Ketoglutarate			−	+
Malate			−	+
Acetate			−	+
Succinate			−	−
Glucose 6-phosphate			−	−
Ribose			−	−

described from electron microscopic studies by Dvorak *et al.* (1985) in the mouse. Stores were low in the oocyte but began to increase during fertilization and rose steeply up to the eight-cell stage; after that, the level was maintained until the blastocyst stage and then decreased in eggs recovered from the uterus but not from those cultured *in vitro* (Edirisinghe and Wales, 1984). The difference is evidently attributable to the oxygen tension commonly maintained in the culture atmosphere (Khurana, 1987) – if held at not more than 5%, the difference between glycogen levels in cultured and recovered embryos disappeared. Evidently glycogen cannot be utilized by the egg before the eight-cell stage. Changes in the volume of lipid droplets in the cytoplasm may indicate a function as an alternative energy source (Dvorak *et al.*, 1985).

Attention has recently been directed increasingly to the question of the stage at which the embryonic genome takes over control of protein synthesis. In the mouse, this is evidently the two-cell stage (Flach *et al.*, 1982; Bolton *et al.*, 1984), but later in other species: in cattle, the four-cell stage (Barnes, 1988) or the eight- to 16-cell stage (Frei *et al.*, 1989); in man, the four- to eight-cell stage (Braude *et al.*, 1988); in sheep, the eight- to 16-cell stage (Crosby *et al.*, 1988). King *et al.* (1988) point out that activation of the embryonic genome coincides with the dispersal of the nucleolar precursor body in the cells of the embryo. They consider morphological changes in the nucleoli of embryonic cells to provide a useful indication of the activity of certain gene loci, and therefore of the onset of embryonic transcription.

The activities of various enzymes have been studied in the early developmental stages of the mouse, rat and rabbit (see Brinster, 1972). Major differences have been recorded between enzyme activities and also variations in the occurrence of enzymes at different stages, but little is known of the significance of these observations. Lactate dehydrogenase is conspicuous for its great abundance at the start of development in the mouse embryo; there was found to be about 10 times as much in the oocyte as in skeletal muscle, the amount later diminishing, and some 70 times as much in the cleaving eggs of the mouse as in those of the rabbit and man. The enzyme in the mouse eggs had an electrophoretic isozyme pattern of type H and at implantation additionally of type M. Again in the mouse, hexokinase was detected at low levels in the oocyte, increasing in activity about sevenfold at the time of implantation, and indications were that it is important in regulating glucose availability. Certain other enzymes present in the pre-implantation stages are known to have a genetic significance and will be discussed later under 'Protein synthesis and gene action' (section 2.2.10).

In the mouse blastocyst, the cavity remains small, about 0.4–0.45 nl; by contrast, the rabbit blastocoele enlarges from about 2 nl to 66 nl at implantation, almost entirely by the intake of water. Other substances are also passed into the cavity, some evidently originating in the bloodstream, though there are distinct differences between the two fluids. One day before implantation in the rabbit, the content of protein and glucose is lower and that of potassium and bicarbonate higher in the blastocoele fluid, but in the next 2 days the composition comes to resemble serum more closely, due to increasing passage to and from the maternal bloodstream. In addition, the phosphorus content doubles and that of chloride increases about threefold. (Further details are given by Brambell and Hemmings, 1949; Lutwak-Mann, 1954, 1959, 1960; Wiley, 1984; Pemble and Kaye, 1986.)

The mechanisms whereby water and ions are taken up into the blastocoele in the rabbit have engaged a good deal of research attention. Some of the first persuasive evidence that there was active transport of ions came when Cross and Brinster (1969) and Gamow and Daniel (1970) reported that a transtrophectoderm potential difference could be

demonstrated in 5½- to 6-day blastocysts, and that its persistence depended on a supply of energy and could be terminated by anoxia or treatment with certain metabolic inhibitors. There remained the question whether ion transport depended on electrical or chemical potential differences across the intervening membrane, or whether transport was effected by an active agent in the membrane. That the latter explanation was preferable was shown by Cross (1973), who applied the Ussing–Zerahn short-circuit technique to 6-day rabbit blastocysts; the results strongly supported active membrane transport of Na^+ and Cl^-, and Cross maintained that this movement of ions would adequately account for the fluid accumulation in the blastocoele. That the 'sodium pump' (Na^+, K^+-ATPase) is the agent in the membrane responsible for Na^+ and Cl^- transport emerges from the work of Borland *et al.* (1967), who used ouabain to inhibit the enzyme. Transport of bicarbonate, which exists in relatively high concentration in blastocoelic fluid, is evidently dependent on a similar mechanism. Hydrogen ions move freely across the membrane without assistance.

Robinson *et al.* (1990) claim to be the first to examine the mechanism of glucose transport across the trophectoderm of rabbit blastocysts; this they maintain is achieved by a sodium-independent system which is unaffected by progesterone, mifepristone, prostaglandins, insulin or cAMP.

Important questions relating to the role of the oviduct in the maintenance of embryos have been investigated by Gandolfi *et al.* (1989). Macrophages cultured with mouse blastocysts have been shown in ultrastructural studies to enter the cytoplasm of trophectoderm cells and to pass between ICM cells; there was no evidence that they exerted any cytotoxic effect (Tachi *et al.*, 1985).

Belling *et al.* (1985) have found that 5- to 6-day rabbit blastocysts can conjugate and actively metabolize diethylstilboestrol, and infer that there are at this stage specific enzyme activities, of which one, monooxygenase, is not expressed in the adult animal. Other aspects of metabolism in cleaving eggs and blastocysts are discussed under 'Recovery, culture, storage and transfer' in section 2.4.1.

2.2.10 PROTEIN SYNTHESIS AND GENE ACTION

The egg during fertilization has a high content of RNA, but this is inherited from the oocyte ('long-lived RNA') and there appears to be no RNA synthesis. Some synthesis occurs at the two-cell stage and then rapidly increases (in the mouse, rabbit and hamster). At the four-cell stage, preponderance of radiolabel over the nucleoli supports the idea that ribosomal RNA (rRNA) synthesis is occurring at this time. Messenger RNA (mRNA) and transfer RNA (tRNA) are also synthesized from the two-cell stage onwards. When development reaches the blastocyst stage, the rates of synthesis of rRNA, mRNA and tRNA have reached levels seen in adult cells (though uterine flushings have an inhibitory effect on RNA synthesis – Weitlauf, 1976).

Protein synthesis has been found to occur in the egg during fertilization (controlled solely by maternal genes), and from then onwards the incorporation of radiolabelled amino acids continues. Between the two-cell and eight-cell stages, the types of protein synthesized change greatly, though those synthesized at the two-cell stage can still be detected in the morula. Proteins specific to certain tissues are first seen at the morula stage. (Reviews on these topics have been published by: Epstein, 1975; Sherman, 1979; Magnuson and Epstein, 1981; Johnson, 1981; McLaren, 1982; Pratt *et al.*, 1983; Johnson *et al.*, 1984; Jackson, 1989.)

On the other hand, Norris *et al.* (1985), characterizing proteins in the cleaving eggs of four species (mouse, rat, hamster and gerbil), found great similarity in the later stages but

striking differences between the species at the one-cell stage. They surmised that the distinctive early protein synthesis on maternally derived templates has the function of maintaining species integrity. At later cleavage stages, the proportion of RNA synthesis controlled by the embryonic genome increases steeply throughout cleavage, so that at the eight-cell stage (or even the six-cell stage) it represents the total. From this point on, maternal genes no longer determine protein characters, and at the blastocyst stage the level of RNA synthesis is equal to that seen in the adult cell. Protein synthesis presents a more complex picture, actual patterns showing the greatest change between the one- and two-cell stages, but synthesis dependent at the two-cell stage on embryo mRNA templates seems to be limited to one protein, β_2-microglobulin. At the four-cell stage, β-glucuronidase appears, and between the eight- and 16-cell two more enzymes, hypoxanthine–guanine phosphoribosyl transferase (HPRT) and glucose phosphate isomerase, and two distinctive proteins, the H-Y antigen and non-H-2 alloantigens. HPRT deficiency causes the Lesch–Nyhan syndrome in the human subject, and the enzyme is also of special interest in that its determining gene is located on the X chromosome. The antigens are important because they are expressed on the surface of cells and are fairly readily detectable.

Evidence of zygote genome expression is also forthcoming from observations on the time of death attributable to lethal mutations carried by the paternal genetic complement. Thus mutants t^{12} and t^{w32} begin to show effects on development at the two-cell stage and cause death of the morula. Homozygotes for the albino locus deletion c^{25H} do not develop beyond a point between the two- and seven-cell stages. (For detailed reviews, see Mintz, 1974; McLaren, 1976a, 1982; Schultz and Tucker, 1977; Adamson and Gardner, 1979; Johnson, 1981; Magnuson and Epstein, 1981; Bolton *et al.*, 1984.) The question whether or not the paternal genome could be dispensed with altogether, in the development of the new individual, is dealt with later under 'Induction of parthenogenesis'(section 2.4.4).

Mitochondria in the developing egg are evidently all maternal in origin; in many animals, sperm mitochondria are released into the egg cytoplasm during early cleavage, but soon show signs of degeneration (Szollosi, 1965, 1976). The wholly maternal origin of these bodies makes it possible to relate individuals to one another by restriction enzyme mapping of the mitochondrial DNA, and study of mtDNAs from 147 individuals from African, Asian, Australian, Caucasian and New Guinea populations led to the conclusion that all could have been descended 200 000 years ago from a single progenitor, probably of African origin (Cann *et al.*, 1987).

Further information on the early effects of the paternal genome in development comes from investigations on experimental hybridization. Some hybrids are, of course, fully viable, but in other instances, though fertilization occurs, development fails before implantation. Insemination of the domestic rabbit with semen from the cottontail rabbits *Sylvilagus floridanus* and *S. transitionalis* resulted in apparently normal cleavage up to the morula stage but no further (Chang and McDonough, 1955; Chang, 1960). A similar experimental series with rabbits inseminated with semen from the hare *Lepus europaeus* yielded blastocysts but no implantation (Adams, 1957; Chang and Adams, 1962). When rabbits were inseminated with the semen of the hare *L. americanus*, there were essentially the same findings (Chang *et al.*, 1964; Chang, 1965). (See also the review by Chang and Hancock, 1967.) These losses of cleaving eggs and blastocysts could presumably have been due to some form of chromosomal imbalance, since in all instances the gametes came from animals with different chromosome number and constitution, but aneuploidy *per se* is not necessarily lethal.

One of the most successful hybrids is the mule [2n=63; *nombre fondamentale* (NF)=99], whose parents are the horse *Equus caballus* (2n=64; NF=94) and the donkey *E. asinus* (2n=62; NF=104), and other examples are listed by Gray (1954). Probably the most systematic enquiries into the effects of aneuploidy were those of Gropp *et al.* (1970, 1976), who crossed the house mouse with the tobacco mouse *Mus poschiavinus*. All house mouse chromosomes are acrocentric, while those of the tobacco mouse include seven metacentrics. The hybrid males had high rates of non-disjunction in spermatogenesis, and when these were back-crossed with female house mice the resulting conceptuses showed various forms of monosomy and trisomy. Monosomy was the more lethal, early development regularly failing to pass the blastocyst stage.

2.3 DEVELOPMENT IN MONOTREMES AND MARSUPIALS

2.3.1 MONOTREMES

In this order of mammals, the processes of egg maturation, fertilization and cleavage have many points of similarity with corresponding events in Sauropsida; original observations are not particularly numerous but a coherent picture is emerging, and the subject has been well reviewed by Boyd and Hamilton (1952), Tyndale-Biscoe (1973), Hughes (1977, 1984), Luckett (1977), Hughes and Carrick (1978) and Tyndale-Biscoe and Renfree (1987) (the last named is a major work with a great deal of useful information on monotremes, though the title names only marsupials).

During fertilization (which is still in progress when the egg reaches the uterus), the cytoplasmic component of the egg takes the form of a flat germinal disc lying upon a large yolk body (see Figure 14.6 in the third edition of *Marshall*), much as in the bird egg. After syngamy, the first cleavage furrow divides the germinal disc into two portions, which are commonly a little unequal in size, and the second furrow crosses the first approximately at right angles. The products are referred to as 'cells' despite the persistence of cytoplasmic continuity between them (the distinctive feature of meroblastic cleavage) as well as on the surface of the yolk body. The third cleavage involves the formation of two furrows, on either side of and roughly parallel to the first, thus dividing the disc into eight cells, regularly arranged but varying in size. The fourth and fifth series of furrows are less regularly oriented and produce groups of 16 and 32 cells with increasing disarray (see Figures 14.7 and 14.8 in the third edition of *Marshall*). Cleavage planes also divide cells into more central and more peripheral portions, thus forming two or three layers of cells, a group of which then become separated by a fluid-filled space from the layer of cytoplasm immediately covering the yolk body; this group forms the 'blastoderm'. The cytoplasmic layer on the yolk surface contains many nuclei and constitutes a syncytium, which spreads to enclose eventually the whole yolk body. The space beneath the blastoderm is termed the 'blastocoele' though not strictly homologous with the eutherian blastocoele. With further cell division, a superficial layer of cells of the blastoderm becomes identified as the embryonic ectoderm, the subjacent layer forming the endoderm. The cells at the periphery of the blastoderm multiply rapidly, spreading over the syncytial layer and forming the 'trophoblast' or 'epiblast'; and eventually, the two layers totally enclose the yolk body and thus the bilaminar blastocyst is established. The mural cells of the blastocyst develop tight junctional complexes and the blastocyst absorbs further fluid and so swells greatly. The ovulated egg was only some 3.5–4.0 mm in diameter, but the blastocyst soon achieves a diameter of 12 mm or more.

While the egg passes along the oviduct and into the uterus (a destination thought to be

Table 2.3 Diameters of ovulated marsupial oocytes

Species	*Vitellus* (μ)	*Overall* [a] (μ)	*Reference*
Dasyurus viverrinus	240	300	Hill (1910)
Didelphis virginiana	135	750	Hartman (1916)
Sarcophilus harrisii	210	300 (435[b])	Hughes (1982)
Trichosurus vulpecula	229	290	Hughes and Hall (1984)

[a]Including zona pellucida, mucoid layer and shell membrane.
[b]After formation of fluid-filled perivitelline space.

reached within 24 h of ovulation), it receives a thin layer of glycoprotein and a shell membrane; next a thick shell is deposited. The shell has been shown to be porous and allow passage of nutrients and gaseous exchange; this facility is important in view of the very limited reserves originally present in the yolk body, which is much smaller than the yolk of an egg of a bird of similar size. The fluids and essential metabolites that are absorbed by the late blastocyst and developing embryo derive from the uterine tissues, which are at this time in a congested and secretory state, under the control of progesterone (Hughes and Carrick, 1978). By contrast, some members of the Sauropsida, such as viviparous snakes and lizards, have well-developed chorioallantoic placentae (see Chapter 15 in the third edition of *Marshall*). Nevertheless, the monotreme embryo possesses a specialized membrane active in metabolic exchange, and there is a functional (though not tactile) relationship with the maternal tissues; on this basis, the monotreme can be said to possess a placenta (choriovitelline, in this case) during its intrauterine existence. The duration of intrauterine life is about a month.

Intrauterine development reaches approximately the 18-somite neurula stage in the platypus and the 19-somite stage in echidna. Up to this time, the only extraembryonic fetal membrane formed is the yolk sac, which is still non-vascularized. Vascularization occurs soon after laying, and then the allantois develops rapidly and in turn becomes vascularized. Eventually, choriovitelline and chorioallantoic composite membranes show about the same degree of development.

The platypus lays its eggs apparently directly into the nest, while the echidna seems to provide pouch accommodation until the young develop their sharp spines. Hatching occurs about 10 days after laying in the echidna (Griffiths *et al.*, 1969), but the period in the platypus is not yet known.

2.3.2 MARSUPIALS

The eggs are distinctly larger than those of eutherian mammals – figures available for four species are set out in Table 2.3. For comparison, the range of diameters of the vitellus alone in eutherians is about 60–180 μm, the largest being that in the sheep egg (Austin, 1961), the overall diameter including the zona pellucida being 200 μm.

The vitellus of the *Dasyurus* egg contains a distinctive body which has been termed a 'yolk mass'; it becomes separate after the first cleavage and is later enclosed within the cavity of the blastocyst. The relatively large size of marsupial eggs in general is commonly attributed to the presence of a plentiful supply of yolk, existing in the dispersed state, the *Dasyurus* egg being exceptional with its striking aggregate, but the deutoplasmic nature of this material is still only hypothetical. A similar 'yolk body' materia-

lizes after the second cleavage division in *Antechinus stuartii* and *Sminthopsis crassicaudatus* and *S. macroura* (Selwood and Young, 1983; Selwood, 1987). Unlike the eutherian egg, the marsupial egg at ovulation is not surrounded by a cumulus oophorus of follicle cells. Sperm entry occurs in the oviduct and fertilization is still in progress when the egg reaches the uterus, which it does within 24 h of ovulation. After sperm entry, the egg receives a thick coating of mucopolysaccharide, homologous with the mucin layer of the rabbit egg, and in addition a keratinous shell membrane which has no equivalent in eutherian eggs, but resembles the corresponding investment in monotreme eggs.

Cleavage results in the formation of increasing numbers of blastomeres, as in eutherians, but a clear difference soon appears in that in marsupials the blastomeres become separated, taking up positions around the periphery. In this manner, in due course, a unilaminar blastocyst is formed, without the intervention of a morula stage or the segregation of an inner cell mass. (Successive stages in the cleavage of the *Sarcophilus* and *Sminthopsis* eggs have been described and illustrated by Hughes, 1982, and Selwood, 1987.) The cells making up the wall of the blastocyst are attached to one another by tight junctional complexes, much as in the eutherian blastocyst, an arrangement permitting control over the passage of water and solutes (as discussed earlier in section 2.2.4). The cells at this stage of the unilaminar marsupial blastocyst are apparently identical, so that there is no obvious way of recognizing the presumptive embryonic area; collectively they constitute the 'protoderm'. At about the 60-cell stage, the blastocyst starts to expand, and indications are that, though development to this point can occur on endogenous resources, expansion and differentiation require the contributions provided by the uterine secretions.

The next step in differentiation involves the detachment of some of the cells in one hemisphere, which at first lie freely in the blastocoele but then come to constitute a layer of loosely connected cells under the protoderm; in this way the endoderm develops, conforming to a pattern like that in the avian egg, but unlike that in the eutherian. Part of the protoderm then differentiates into an oval plate of cuboidal cells which later becomes the medullary plate of the embryo. A primitive groove with Hensen's node then appears in the medullary plate, and it is from the primitive groove that mesoderm cells are generated and become distributed between protoderm and endoderm. Subsequent developmental stages include the formation of the notochord and neural tube, and later the spinal cord, brain vesicles and the somites. During the course of these events, the extraembryonic membranes are being formed, and large differences in pattern are discernible in different species. As in eutherians, two membrane structures can be important in the establishment of a system allowing uptake of nutrients by the embryo, gaseous exchange and elimination of wastes; these are the yolk sac and allantois, though in order to achieve close relations with the maternal environment some collaboration with the chorion is also needed, and thereby choriovitelline and chorioallantoic placentae are formed. These composites vary in their extent of vascularization, and this in turn depends upon the range of development of the mesoderm at an earlier stage. In marsupials, the mesoderm stops short of investing more than about half of the wall of the embryonic vesicle, so that the regions of placentae that become trilaminar and vascularized are rather limited. In *Didelphis* and *Dasyurus*, the only region to become thus developed is part of the choriovitelline membrane, whereas in *Phascolarctos* and *Perameles*, the honour is shared between the two composites. The prevailing view is that vascularized regions are mainly concerned with respiration and the non-vascularized with nutrition.

Differences also exist among marsupial species in the degree of intimacy that is achieved between embryonic and maternal tissues. The shell membrane may persist intact through much of intrauterine life in some species, as for example in *Trichosurus vulpecula* in which Hughes and Hall (1984) found that the membrane was not lost before the two-thirds point of the 17.5-day gestation period. But a placenta involving invasion of the maternal tissues by the fetal is formed in other species, as distinctively shown in *Perameles* (see Amoroso, 1952).

Both lactational and seasonal diapause are well known in marsupials. The former was demonstrated in the quokka *Setonyx brachyurus* and the tammar wallaby *Macropus eugenii* by Sharman (1955), the presence of pouch young inhibiting implantation of the uterine blastocyst that had arisen from a post-partum mating. Implantation followed removal of the pouch young; and the same mechanism appears to exist in the euro *M. robustus* and the red kangaroo *M. rufus*. Here, too, artificial control of photoperiod can produce the same result.

For further information on the pre-implantation development of marsupials, the reader is referred to reviews by Tyndale-Biscoe (1973), Hughes (1977, 1984), Renfree (1978,1982), Tyndale-Biscoe and Renfree (1987) and to Chapter 7 in this volume.

2.4 EXPERIMENTAL MANIPULATION

2.4.1 RECOVERY, CULTURE, STORAGE, TRANSFER

Recovery of cleaving eggs from the oviduct and blastocysts from the uterus is commonly achieved by flushing with a balanced salt solution or heat-treated homologous serum or a mixture of the two (Table 2.4). For this purpose, phosphate buffering is appropriate for maintaining a pH in the region of 7.0–7.5. In expendable animals, the oviduct is removed post mortem, straightened out if necessary by dissection, and then flushed by inserting a blunted hypodermic needle with syringe attached into the uterine ostium, the emerging fluid being collected in an embryological watchglass. This procedure is satisfactory for the rabbit; with the rat and mouse, flushing is easier done in the opposite direction, the needle being inserted into the infundibular end of the oviduct. In larger animals, and where the animal is to be preserved, laparotomy is performed and the ovarian end of the uterus is clamped off, the flushing medium being injected into this region and forced along the oviduct.

To obtain blastocysts from the uterus, dissection is usually depended on in expendable animals, but in others (generally farm animals, but rather similar methods are used in the human subject) a non-surgical procedure is employed. This involves the use of a long plastic catheter, of which two designs are about equally popular. In both, there is an inflatable bulb within a short distance of the end that is to be passed into the uterus; when the bulb is expanded with air, a region at the ovarian end of the uterus is closed off so that it will retain injected fluids. Accordingly, in one design of catheter, there are two lumina, through one of which air is forced for pumping up the bulb, and through the other flushing fluid is injected and later drained out, carrying the blastocysts with it. The other design of catheter has three lumina, one for bulb inflation as before, a second for fluid injection and the third for fluid return (which with this device can be simultaneous with injection). (Detailed descriptions of these techniques are given by Croxatto *et al.*, 1972; Hunter, 1980; Brackett *et al.*, 1981; Buster *et al.*, 1983; Trounson, 1983; Bavister *et al.*, 1984; Betteridge, 1986; Brambati and Tului, 1990.) Loskutoff *et al.* (1990) describe the non-surgical transcervical recovery of embryos from the uterus of the suni (*Neotragus moschatus zuluensis*), a small East African antelope, and give references to similar work on other African ungulates.

Culture of cleaving eggs, morulae and blastocysts has been carried out in several different kinds of receptacles, including small plastic Petri dishes, test tubes and agglutination tubes, but some prefer embryological watchglasses, Carrel flasks or capillary tubes. The composition of the culture medium is critical in respect of pH, osmolarity, metabolizable nitrogen and energy sources, certain ions and a macromolecular component such as serum albumin. Sometimes, additions of fetal calf serum or human umbilical cord serum appear necessary. Variations in the properties of the medium suit different species.

Rabbit cleaving eggs were the first to be cultured through to blastocysts (Lewis and Gregory, 1929), for which a mixture of 50% or more of serum in balanced salt solution is sufficient, though a uterine secretory component may be needed for full blastocyst expansion (see section 2.2, The cleaving eutherian egg and the blastocyst).

Most attention, however, has been given to determining the precise cultural requirements for mouse pre-implantation development. Hammond (1949) observed that mouse eggs would proceed to the blastocyst stage from the eight-cell (but not earlier) in a simple saline mixture containing hen egg-white. Whitten (1956), however, found this medium to be unstable in air, the pH rising rapidly owing to loss of CO_2 from the egg-white. He therefore used a Krebs–Ringer bicarbonate medium in stoppered tubes, but the addition of egg-white was still necessary, because of an essential but unidentified non-dialysable component. Bovine serum albumin came to be preferred to egg-white and, with this, eggs would consistently pass from the eight-cell to

Table 2.4 Milestones in the history of oocyte and egg manipulation—publications on research that resulted in the birth of young (Dates in parenthesis apply to reports of observations that were the first of their kind, but did not include the birth of young.)

Rabbit						
IVF			1959[a]			
Culture		1947[b]				
Transfer	1891[c]					
Mouse						
Oocyte cryopreservation					1977[d]	
Oocyte maturation					1977[d]	
IVF				(1968)[e]	1970[f]	
Culture		(1949)[g]	1958[h]			
Embryo cryopreservation				1971[i]		
Transfer		1942[j]				
Man						
Oocyte cryopreservation						(1986)[k]
Oocyte maturation				(1965)[l]		
IVF				(1969)[m]		
Culture					(1970)[n]	
Embryo cryopreservation						(1980)[o]
						1985[p]
Transfer					1978[q]	

References: [a]Chang (1959); [b]Chang (1947); [c]Heape (1891); [d]Whittingham (1977); [e]Whittingham (1968); [f]Mukherjee and Cohen (1970); [g]Hammond (1949); [h]McLaren and Biggers (1958); [i]Whittingham (1971); [j]Fekete and Little (1942); [k]Chen (1986); [l]Edwards (1965); [m]Edwards *et al.* (1969); [n]Edwards *et al.* (1970); [o]Whittingham (1980a); [p]Cohen *et al.* (1985); [q]Steptoe and Edwards (1978).

the blastocyst. Development from the two-cell stage to the blastocyst would occur if the glucose in the medium were replaced by lactate and the space above flushed out with 5% CO_2 in air (Whitten, 1957; see also Whitten and Biggers, 1968, and Whitten, 1971). Development through from the one-cell zygote to the blastocyst, however, has been found unlikely to occur in chemically defined media, owing to the now well recognized two-cell block shown by many strains of mouse. The are also, however, non-blocking strains, which indicates that blocking could be attributable to a deficiency of some kind, an inference supported by the observation of Muggleton-Harris *et al.* (1982) and Pratt and Muggleton-Harris (1988) that the defect can be overcome by transfer of cytoplasm. Recent observations by Nasr-Esfahani *et al.* (1990) suggest that the cytoplasm in the non-blocking strains contains chelating agents that counter free radicle production by traces of iron responsible for the effect. More recently, Lawitts and Biggers (1991) have cultured pronuclear stage mouse eggs to the blastocyst stage in media whose composition was determined by 'simplex optimization', which examines a large number of variables simultaneously.

The importance of the composition of the atmosphere over the culture medium was pursued by Quinn and Harlow (1978), who noted that the proportion of mouse blastocysts developing, as well as the number of blastomeres per blastocyst, was increased if the oxygen content were reduced to between 2.5 and 5%. Later, Khurana (1987) reported that the turnover in acid-soluble glycogen in mouse morulas and blastocysts increased steadily as the oxygen concentration was reduced from 20% to 1%, the trend being accompanied by increase in rate of cell division.

That there were advantages in co-culturing eggs with other cells was reported by Cole and Paul (1965), who obtained enhanced development of mouse eggs if cultured with irradiated HeLa cells. Kuzan and Wright (1982) found that bovine blastocysts showed improved rates of hatching and attachment to the endometrium if previously incubated with a layer of 'feeder' cells. Wiley *et al.* (1986) obtained better development of mouse eggs when they were cultured in larger groups. Heyman *et al.* (1987) reported improved rates of development of cow and sheep eggs when cultured with trophoblastic vesicles and serum; the method also overcame the eight- to 12-cell stage development block, and cleavage proceeded to 16 cells or more. Benefit was also noted following co-culture with oviduct tissue (Eyestone and First, 1989; Eyestone *et al.*, 1991). Clearly there are components of a successful culture medium yet to be identified. An essential property would seem to be large molecular weight, as suggested by the earlier observations of Cholewa and Whitten (1970) that mouse eggs would develop normally from two-cell stage to blastocyst in the absence of any fixed nitrogen source, including albumin, provided the medium contained polyvinylpyrrolidone. Hamster embryos were found to differ greatly from mouse embryos in their energy substrate needs by McKiernan *et al.* (1991), who successfully cultured them from one-cell stage to blastocyst.

Rat eggs proved more difficult subjects for culture than mouse eggs, but rat blastocysts have nevertheless been maintained through to the mid-somite stage with beating 'heart' (Hsu, 1971), and even to the limb-bud stage of development (Chen and Hsu, 1982). Rabbit one-cell eggs have been grown in culture to the hatching blastocyst stage in a defined protein-free medium by Carney and Foote (1991), who claim this to be the first such success. Pig embryos could be grown in culture from the one- to two-cell stage to the morula or blastocyst in 45–60% of trials if glucose or glutamine (or the two together) were present in the medium (Petters *et al.*, 1990). The success with glutamine demon-

strates that an amino acid can be used as the sole source of energy for full preimplantation development. The metabolic availability of glutamine to early mouse embryos was confirmed by Chatot *et al.* (1990), who maintain that the amine plays an important role in sustaining the earliest stages of development. Bovine embryos have been grown from the one- to two-cell stage to morulae and blastocysts both in rabbit oviducts and in co-culture with bovine oviduct epithelial cells suspended in a simple medium (Ellington *et al.*, 1990), or epithelial cells suspended in TCM-199 medium (McCaffrey *et al.*, 1991); also in a mixture of synthetic oviduct fluid and fetal calf serum or human serum, under an atmosphere of 5% oxygen, by Fukui *et al.* (1991). Eggs from the rhesus monkey have been successfully cultured after fertilization *in vitro*, and viable young have been obtained after transfer to recipients (Figure 2.6) (Bavister *et al.*, 1983; Boatman, 1987). For the culture of human eggs from the one-cell stage through to the morula/blastocyst, good results have been obtained with Ham's F-12 medium (Muggleton-Harris *et al.*, 1990). More recently, improved blastulation and implantation rates after co-culture of embryos with tubal ampullary epithelial cells have been reported (Bongso *et al.*, 1992). The embryos of two dasyurid marsupials were cultured in cleavage and blastocyst formation by Selwood (1987). (Publications appearing up to 1958 on the culture of eggs and blastocysts were tabulated by Austin, 1961; for more recent reviews see Gwatkin, 1966; Bindon, 1971; Daniel, 1971, 1978; Brinster, 1972; Hunter, 1980; Edwards *et al.*, 1981; Bavister, 1986; Greve *et al.*, 1989.)

Storage of mammalian embryos at average refrigerator temperatures is of limited value because of loss of viability within a few days. By contrast, storage at temperatures well below freezing has proved highly successful. Striking results were obtained first with the low-temperature preservation of spermatozoa, blood cells and various tissues (see A.U. Smith, 1961, for a review of earlier work), and these greatly stimulated efforts to do the same with oocytes, cleaving eggs and blastocysts. It was, however, several years before Whittingham (1971) was able to report encouraging results in the mouse; he held 8-cell eggs and early blastocysts at −79°C for 30 min, with only polyvinylpyrrolidone as cryoprotectant. With the aid of dimethylsulphoxide (DMSO), it proved possible to store eggs at −196 and −269°C for several weeks and yet obtain the subsequent birth of a high proportion of normal young (Whittingham *et al.*, 1972). Some variables in egg survival were also studied by Wilmut (1972). Later, Whittingham (1974, 1975, 1976) reported that the freeze storage of eggs was a reliable and economical way of maintaining a large number of inbred mouse strains, and that oocytes could be fertilized *in vitro* after storage and, on transfer to recipients, would develop into normal young at birth. More recently, Rall and Fahy (1985) recommended a cryoprotectant mixture for mouse eggs consisting of

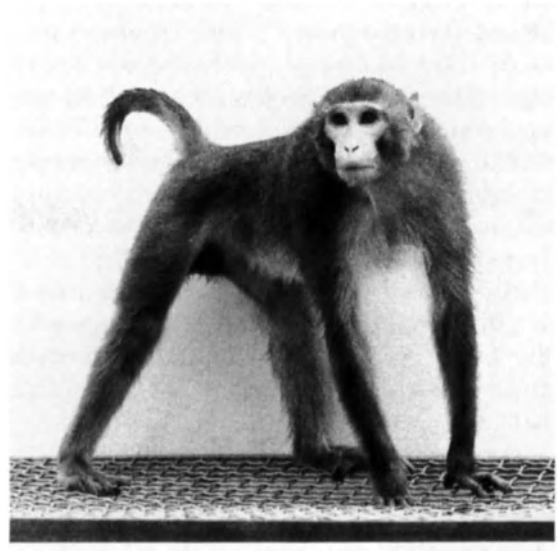

Figure 2.6 'Petri', a rhesus macaque, the first non-human primate to develop from *in vitro* fertilization and embryo transfer, at 3 years and 5 months (see Bavister *et al.*, 1984). (Courtesy of B.D. Bavister, Department of Veterinary Science, University of Wisconsin, Madison, USA.)

dimethylsulphoxide, acetamide, propylene glycol and polyethylene glycol. Kasai *et al.* (1990) obtained very high rates of survival of mouse embryos frozen after being equilibrated in a medium containing ethylene glycol or glycerol. Shaw *et al.* (1991) have recommended rapid freezing of two-cell mouse embryos in just DMSO, but at a concentration of 4.5 M, lower concentrations being associated with reduced embryonic viability and chromosome damage.

Sheep and cow embryos have also been cryopreserved and later transferred to recipients, those of the cow often on a very large scale (Wilmut and Rowson, 1973; Willadsen *et al.*, 1974, 1976, 1978; Betteridge, 1977, 1986; Polge, 1978; Hunter, 1980; Seidel *et al.*, 1983; Wright, 1985), but not very satisfactory results have yet been obtained with the pig. Even 'half' and 'quarter' cattle embryos have been preserved by freezing (Lehn-Jensen and Willadsen, 1983). The freezing of horse embryos has been reported by Yamamoto *et al.* (1982) and Czlonkowska *et al.* (1985). Human cleaving eggs and blastocysts can be cryopreserved (Whittingham, 1980a; Trounson and Mohr, 1983; Mohr *et al.*, 1985) and develop to birth after transfer (Cohen *et al.*, 1985); this field has been reviewed by Ashwood-Smith (1986), and see also the 'Symposium on Embryo Freezing' (mouse, baboon, man) in the *Journal of In Vitro Fertilization and Embryo Transfer* (1986), Vol. 3, pp. 1–61. More recently, Fugger *et al.* (1991) and Hartshorne *et al.* (1991) reported on their experiments with the freeze-preservation of human embryos and subsequent establishment of pregnancies.

Transfer of cleaving eggs probably began with Heape's (1891, 1897) classical experiment of inserting two four-cell eggs from an Angora rabbit into the oviduct of a mated Belgian hare; two young Angora rabbits were among the litter of six born later. Heape records how he made the transfer without the use of a fluid vehicle, picking up the eggs 'on the point of a spear-headed needle'. Since then a very large number of transfers has been made under a variety of circumstances. Literature surveys have been published by Adams and Abbott (1971) and Adams (1975, 1977), the subject has been reviewed by Austin (1961), Chang and Pickworth (1969), Dickmann (1971), Polge (1978), Hunter (1980) and Trounson (1983) and a symposium volume edited by Adams (1982) has been published. Animals used as donors and as recipients include the rabbit, hare, mouse, rat, hamster, guinea pig, ferret, mink, cow, sheep, goat, pig, horse, donkey, man, baboon, rhesus monkey, tammar wallaby and quokka (list compiled by Polge, 1978, and see also Oguri and Tsutsumi, 1982). To these can now be added: zebra (mare recipient: Betteridge, 1986), water buffalo (intraspecific: Drost *et al.*, 1983), rhesus monkey (IVF and transfer: Bavister *et al.*, 1984), squirrel monkey (IVF and transfer: Kuehl *et al.*, 1984), marmoset (intraspecific: Hearn, 1984), and embryos of Przewalski's horse and common zebra (*Equus burchelli*) transferred to domestic horse and donkey (Hearn and Summers, 1986; Summers *et al.*, 1987).

Oocytes and cleaving eggs are commonly inserted into the oviduct at laparotomy. In the human, however, less invasive techniques are preferred. Unfertilized, pronucleate and cleaving eggs are transferred to the outer fimbriated ends of the fallopian tubes translaparoscopically (Asch *et al.*, 1984; Yovich *et al.*, 1987; Balmaceda *et al.*, 1988). Several procedures have also been devised to visualize and access the tubes transvaginally using catheters, including radiography (Platia and Krudy, 1986), hysteroscopy (Confino *et al.*, 1986) and ultrasound (Jansen and Anderson, 1987). Pregnancies have been established in human subjects using transvaginal tubal transfer of gametes and embryos with (Bustillo *et al.*, 1988; Jansen *et al.*, 1988) and without (Bauer *et al.*, 1990) ultrasound guidance.

Embryos of various stages of development can also be transferred directly into the

uterus. Surprisingly, even uterine transfer of pronucleate eggs has resulted in pregnancies in the human (Ahuja *et al.*, 1985). Although embryos can be introduced surgically, most of the investigators (one of the earliest being Sugie, 1965) prefer non-surgical transfer involving the use of specialized equipment: a fine plastic catheter, outer sheath and instillation syringe, for human subjects (as described by Craft, 1984), or 'French straws' and a Cassou artificial insemination gun for cattle (as described by Betteridge, 1986). Table 2.4 summarizes the chronology of technical advances in the manipulation of oocytes and cleaving eggs.

Embryos have also been deposited in locations other than the genital tract ('ectopic transfer'). Some of the earliest successful transplants were of rat eggs to beneath the kidney capsule (Nicholas, 1942) and of mouse eggs to the anterior chamber of the eye (Fawcett *et al.*, 1947; Runner, 1947), where implantation on the iris often occurred and further development could be observed directly. Later, ectopic transfer was extensively used by Kirby (1960, 1963a,b, 1967), who found that cleaving mouse eggs would eventually develop some measure of placentation, not only in the kidney and spleen but even in the testis. Results showed the low antigenicity of the embryo, for grafts of more advanced tissues are very likely to be rejected. Then Stevens (1970) observed that mouse blastocysts injected under the testis capsule finally became disorganized and formed teratomas and teratocarcinomas, a finding that initiated a new line of research, which is discussed later in this chapter.

2.4.2 ATTEMPTED INDUCTION OF DEVELOPMENTAL ANOMALIES

With the successful induction of fertilization and cleavage *in vitro* of human eggs (Edwards *et al.*, 1969, 1970), and research then directed to obtaining pregnancies and full-term births, concern was widely expressed that the manipulations involved might interfere with normal development and produce birth defects, and so the literature was searched for any evidence to support these fears (Austin, 1973). This revealed that cleaving eggs and blastocysts of several different species had been subjected to wide variations in culture conditions, including large differences in pH and osmolarity, to storage for many weeks at −269°C, to incubation at temperatures up to 40°C, to metabolic inhibitors and stimulants, and to ionizing radiations, and in no instance had there been a significant increase in the incidence of developmental defect among the young eventually born. This remarkable imperturbility is probably attributable to the fact that the cells destined to form the future embryo are undifferentiated in the cleaving egg and blastocyst, so that while traumatic agents may cause the death of cells they cannot affect subsequent genome expression.

Later, Spielmann (1976) reviewed the field again and came to much the same conclusion, pointing out, however, that agents applied to the blastocyst just before implantation might remain in the blastocoele and interfere with morphogenetic processes at later stages. Such damaged embryos, however, almost certainly die before birth and do not contribute to the incidence of birth defects.

Essentially consistent findings were reported more recently by Fabro *et al.* (1984), who treated eight- and 16-cell mouse and rabbit eggs with different concentrations of alkylating agents. In the mouse, methylmethanesulphonate reduced the number of morulas and blastocysts developing in culture and the number of blastocysts 'hatching'. When treated eggs or blastocysts were transferred to recipients, implantation rate showed no reduction and the offspring at birth were free from major congenital defect, though mean birth weight was lower than in the controls. In the rabbit, following treatment with the same agent or with thalidomide or dichlorodiphenyltrichloroethane (DDT), there was a

reduction in birth weights but not in litter size and no congenital defects were observed. The authors inferred that damage was done to the developing conceptuses by the alkylating agents used, but that this was subject to compensatory repair during pregnancy in those that survived.

In human subjects, diabetic mothers have a two- to fourfold higher incidence of malformed infants than normal mothers, but observations in the Chinese hamster showed that spontaneously occurring maternal diabetes had no detectable influence on the cleavage rate of fertilized eggs or on the incidence of pre-implantation embryonic death, gross malformation or hypoplasia (Funaki and Mikamo, 1983).

2.4.3 NUCLEUS REMOVAL AND INSERTION

The pioneering work was that of Briggs and King (1952), following a suggestion made by Spemann (1938), who had observed that, when a fertilized newt egg was constricted by tying a hair about its equator, the cleaving nuclei were at first confined to one side of the knot; after some cleavage divisions, however, a nucleus sometimes migrated through the constricted region and initiated cleavage in the other half of the egg. Whether or not the two halves developed similarly or dissimilarly depended on the position of the constriction relative to the distinctive 'dorsal lip' region of egg cytoplasm, rather than upon the number of cleavages in the two halves. If the knot divided the dorsal lip cytoplasm equally, the two half eggs would produce identical embryos, even if the migrating nucleus had passed through when the cleaving half had reached the 16-cell or even the 32-cell stage. It was surmised that all these early nuclei were developmentally totipotent. Spemann argued that particular interest would attach to the injection of nuclei taken at later stages, after differentiation had occurred, into nucleated one-cell eggs, but in his day the necessary technology was lacking.

Briggs and King (1952) transferred nuclei from the animal hemisphere of frog blastulae to enucleated unfertilized eggs. Most of the treated eggs proceeded to develop normally into complete embryos, and many into normal tadpoles. With nuclei taken from blastulae, early gastrulae and late gastrulae, the proportion of recipient eggs proceeding to normal tadpoles declined correspondingly, suggesting a progressively limiting differentiation, though even nuclei taken from the tissues of adult animals could support extensive development. Best results were obtained with *Xenopus* (see Table 2.5) (Gurdon, 1962; Gurdon and Uehlinger, 1966; and see the review by Briggs, 1977).

Determined efforts have been made to repeat these observations in mammals, but largely in vain. Bromhall (1975), transferring nuclei from rabbit morulae to enucleated oocytes, was able to observe good nuclear development but very limited cleavage. Illmensee and Hoppe (1981) and Hoppe and Illmensee (1982) claimed that nuclei taken from the inner cell mass cells of fertilized or parthenogenetic mouse blastocysts and injected into fertilizing eggs from which the pronuclei had been removed would, in a few instances, permit development to term; but these findings have not been confirmed. On

Table 2.5 The developmental potential of nuclei from tadpole intestinal cells, as described by Gurdon (1968)

*Mono*nucleolated strain of frog	Intestinal epithelial cell nucleus injected into: V
*Bi*nucleated strain of frog	Oocyte, previously irradiated to destroy nucleus. Cultured. Result:
	No cleavage OR Abnormal embryo OR Mononucleolated adult frog

the contrary, McGrath and Solter (1983, 1984a) found that when nuclei from two-cell mouse eggs were injected into enucleated eggs undergoing fertilization, the products developed only to the blastocyst stage, and the use of nuclei from four-cell or eight-cell eggs, or from the inner cell mass cells of blastocysts, was not followed by significant development. Surani (1984) also reported that eight-cell and ICM nuclei were incapable of supporting more than two or three cleavage divisions.

More recently, Willadsen (1986), working with the sheep, found that if the nuclei for transfer were taken from embryos that were themselves the products of nuclear transfer, more extensive development ensued. Prather and First (1990) infer that when lack of significant development after nuclear transfer is repeated, this reflects the effects of asynchronies in donor and recipient cell cycles, and of deficiencies in genomic modification. In addition, however, Willadsen (1986) depended on blastomere transfer (from eight- and 16-cell embryos) as the means for nuclear transfer, achieving in this way development through to birth. Blastomere transfer avoids the problem that damage is done to naked nuclei by manipulative procedures. By these means, other investigators have succeeded in obtaining live-born calves from blastomeres removed from embryos up to approximately the 64-cell stage (see Massey, 1990). Prospects for the production of very much larger numbers of cloned calves are discussed by First (1990), who explains how the embryo developing from an oocyte implanted with a blastomere can in its turn serve as the source of up to about 64 transferrable blastomeres. Prior to such multiplication, embryos can be sexed by immunological methods.

2.4.4 INDUCTION OF PARTHENOGENESIS

Development by parthenogenesis is known in a wide range of organisms, in many of which it represents a normal reproductive process. Spontaneously occurring limited parthenogenetic development has been recorded in several mammalian species (see Kaufman, 1983) and is particularly notable in the ovaries of mice of the LT/Sv strain, in which egg cleavage frequently advances to the blastocyst stage and occasionally somewhat beyond (Stevens and Varnum, 1974). In no mammal, however, is there certain evidence of unaided parthenogenetic development (spontaneous or induced) leading to the birth of viable young (the literature has been reviewed by Suomalainen, 1950; Beatty, 1957, 1967; Austin and Walton, 1960; Tarkowski, 1971; C.F. Graham, 1974; Mittwoch, 1978; Whittingham, 1980a,b; Markert, 1982; Kaufman, 1983; Austin, 1987).

Parthenogenesis may be defined as development of the egg without the involvement of the male in any way. Gynogenesis occurs if a spermatozoon activates an egg but provides no genetic material to the future embryo. In androgenesis, the spermatozoon participates as in normal fertilization but the egg's genetic material is not involved. A few species of non-mammalian animals are known to reproduce by gynogenesis or by androgenesis, but we have very limited information on them. A major reason for continued research interest in this subject is that parthenogenetically and gynogenetically derived animals would possess only maternal genes and would be invaluable for the study of recessives. In addition, the fact that none of these alternatives to fertilization has yet been known to succeed demands an explanation.

Some observations were made on the tendency of unfertilized eggs to undergo apparently normal cleavage, with or without experimental stimulation (Austin, 1949, 1956). By contrast, human follicular oocytes are unusually resistant to parthenogenetic activation (Abramczuk and Lopata, 1990), although activation can occur after micromanipulation of oocytes (Kola *et al.*, 1990). The current phase of research on parthenogenesis may be said to have started with the

reports of C.F. Graham (1970) and Tarkowski *et al.* (1970) on their work in mice. C.F. Graham (1970) induced development simply by removing the cumulus oophorus with hyaluronidase, growing the eggs in culture for a short time and then transferring them to suitable recipients. Tarkowski *et al.* (1970) used electrical shock to stimulate eggs still in the oviduct, and recovered them at a later time or looked for any implantations. Both laboratories recorded a high incidence of activation and some implantation, and occasionally the presence of developmental stages further advanced than those previously described, namely the egg cylinder stage, with some embryos surviving for 9–10 days. Development appeared to be normal as far as it went and so the possibility presented itself that at least a small proportion of parthenogenones might survive to birth and beyond, but as yet and despite diligent efforts such an outcome has not been observed. Interesting possible explanations have been put forward. Recent findings may be summarized as follows:

1. Diploid parthenogenones have been united with cleaving fertilized eggs to make chimeras (entities that are discussed in the next section), and these, on being placed in recipient mice, have developed to term, further investigations revealing that the parthenogenetic line contributed to all tissue classes (Stevens *et al.*, 1977; Surani *et al.*, 1977), and even to fertile germ cells (Stevens, 1978; Anderegg and Markert, 1986).
2. Gynogenetic and androgenetic development has been produced by removing the male or the female pronucleus, respectively, from eggs undergoing fertilization, and restoring diploidy by inhibiting the first cleavage division with cytochalasin B. A few of these embryos of both kinds were claimed to be capable of developing to term (Hoppe and Illmensee, 1977). This report was later said to have been based on technical error (see Newmark, 1983, 1984).
3. After removal of the cumulus and zona pellucida, fusion was induced between oocytes and first polar bodies by treatment with 40% polyethylene glycol or UV-inactivated HVJ virus. The latter treatment yielded two blastocysts developing from 45 oocytes successfully fused with polar bodies (Tachi and Tachi, 1980a).
4. Fusion between paris of denuded oocytes was induced by treatment with inactivated Sendai virus (Soupart, 1980, 1982) or with phytohaemagglutinin followed by polyethylene glycol (Gulyas *et al.*, 1984), and then by a brief treatment with ethanol to increase the frequency of activation. Development in subsequent culture sometimes went as far as the blastocyst stage. When Gulyas *et al.* (1984) transferred blastocysts to suitable recipients, about half implanted and one embryo proceeded to the 14-somite stage.
5. Nuclei were removed from the inner cell mass of blastocysts developing from either fertilized or parthenogenetically activated eggs and injected into enucleated fertilized eggs, and some of the products were said to have developed to term (Hoppe and Illmensee, 1982). This paper was subject to the same qualification as that mentioned in paragraph 2 above.
6. Fertilized eggs were treated so as to induce suppression of the second polar division, and diploidy was restored by removal of one of the female pronuclei or of the male pronucleus. Eggs retaining the male pronucleus often developed to term but all others failed (Surani and Barton, 1983).
7. The two pronuclei of eggs undergoing fertilization were introduced into enucleated parthenogenetic eggs; development then often continued to term (Mann and Lovell-Badge, 1984). Replacing the male and female pronuclei with nuclei from

parthenogenetic eggs, however, did not have this effect.

8. The introduction of a male pronucleus into a haploid parthenogenetic egg was often followed by development to term, but this did not happen when a female pronucleus was inserted (Surani *et al.*, 1984).
9. Eggs undergoing fertilization were treated in two ways: (a) the male pronucleus was removed and replaced by either a male or a female pronucleus or (b) the female pronucleus was removed and replaced by a male or a female pronucleus; development to term only occurred when a male and a female pronucleus were present (Barton *et al.*, 1984).
10. Mouse blastocysts were reconstituted in two different ways: the inner cell mass from a parthenogenetic or a gynogenetic blastocyst was used to replace the ICM in a fertilized blastocyst, or the ICM from a fertilized blastocyst replaced that in a parthenogenetic or a gynogenetic blastocyst. In no instance did development go to term, nor did ICM or trophectoderm cells contribute to the deficient partner. Best results came from blastocysts in which parthenogenetic or gynogenetic ICMs existed within normal trophectoderms – development was rarely less than equivalent to 25 somites and in many instances proceeded to 30 or 40 somites (Barton *et al.*, 1985).
11. Gynogenesis and androgenesis were induced by removal of the male or female pronucleus, respectively, from mouse eggs undergoing fertilization and development was found generally to proceed to two- to 16-cell stages with gynogenesis and two- to four-cell stages with androgenesis. Nuclei from cleaving gynogenetic eggs could replace female pronuclei (but not male pronuclei) in eggs undergoing fertilization, and the product would develop to term; the same outcome would ensue with the replacement of male pronuclei (but not female pronuclei) by nuclei from cleaving androgenetic eggs (Surani *et al.*, 1986).

From these observations (summarized in Tables 2.6 and 2.7) it is inferred that failure of parthenogenones is not attributable to homozygosity and the expression of recessive lethal genes, as has often been suggested, or to a possible 'essential' extranuclear contribution by the spermatozoon, but specifically to some influence conveyed by the male pronucleus.

Surani *et al.* (1984) proposed that the male genome must undergo a distinctive 'imprinting' as to its paternal origin during gameto-

Table 2.6 Parthogenetic eggs have limited developmental potential unless appropriately modifed (Various sources)

Parthenogenone (haploid or diploid)	Blastocyst
+fertilized embryo (chimaera)	Fertile adult, including germ cells
, enucleated, +♂+♀ pn	Term
,+2♂ *or* 2♀ pn	Blastocyst
, +♂ pn	Term
, +♀ pn	Blastocyst
Fertilized egg, triploidized with cytochalasin B	Blastocyst
and ♂ pn removed	Blastocyst
and 1 ♀ pn removed	Term

pn=pronucleus

Table 2.7 Results of replacing pronuclei in fertilizing eggs with nuclei taken from cleaving gynogenetic and androgenetic eggs. See Surani *et al.* (1986)

Fertilized egg, ♂ pn out (gynogenesis),		develops to	2–16 cells
nucleus used to replace	♀ pn	develops to	term
nucleus used to replace	♂ pn	develops to	cleavage only
Fertilized egg, ♀ pn out (androgenesis),		develops to	2–4 cells
nucelus used to replace	♀ pn	develops to	cleavage only
nucleus used to replace	♂ pn	develops to	term

pn=pronucleus

genesis, and that this influence is essential for the proper development of extraembryonic membranes and trophoblast, since these structures are notably underdeveloped in parthenogenones. Eggs with one or two male pronuclei and no female pronucleus also fail to develop to term, the female contribution evidently being essential for some phase of embryogenesis, and again the suggestion is that genomic imprinting may take place during gametogenesis, this time in the ovary (Barton *et al.*, 1984; McGrath and Solter, 1984b; Solter, 1987). The observations support the idea that the cytoplasm of parthenogenetic and fertilized eggs is similar and that it is some deficiency in the genotype of the former that is the reason for failure to achieve full development (Surani *et al.*, 1987). The role of genomic imprinting is discussed further by Jackson (1989).

A possible explanation for some of the observations lies in the need for X-inactivation in the late blastocyst. As first reported by Takagi and Sasaki (1975), X-inactivation in mice is random in embryonic cells but preferentially affects the male X chromosome in those of the extraembryonic tissues. If only female X chromosomes are present, there could be an imbalance affecting development, this state existing in diploid parthenogenones. Kaufman *et al.* (1978), however, found that X-inactivation did in fact occur in more than 50% of the cells, and Rastan *et al.* (1980) showed that a similar proportion existed in the yolk sacs. This explanation, therefore, appears inadequate.

The effects of certain mutations can also differ according to their transmission through maternal or paternal routes. Hairpin-tail (T^{hp}) in the mouse, for example, allows survival in the heterozygote when derived from the father but causes death of the embryo about half-way through pregnancy when derived from the mother. Then again, in the DDK strain of mouse, matings between males and females within the strain are much more fertile than outcrosses of the females with alien males, which result in frequent death of morulae and blastocysts, and again just before the egg cylinder stage. A suggestion is that embryonic death is attributable to maldevelopment of the trophoblast.

In summary, we still lack a working hypothesis for the limited viability of mammalian parthenogenones.

2.4.5 MICROSURGICAL FERTILIZATION

In classic experiments using non-mammalian eggs, Hiromoto (1962) described a system for the mechanical breaching of the zona pellucida using a micropipette which has become the standard for sperm microinjection techniques in mammals. This and subsequent sperm microinjection studies have helped to elucidate the nature of gamete interaction.

Several workers have demonstrated that the injection of motile and immotile spermatozoa, either directly into the cytoplasm of

the oocyte or into the perivitelline space, can lead to pronuclear formation and normal development in mammals (Uehara and Yanagimachi, 1976; Thadani, 1980; Markert, 1983; Mann, 1988). In addition, interspecies fertilization, which would not otherwise have been possible, has been shown to occur after injection of foreign sperm directly into the ooplasm (Thadani, 1980). From these studies, it can be concluded that normal surface interactions between the spermatozoon and the egg are not essential to initiate development.

The ability of the oocyte to decondense sperm is acquired gradually by the egg cytoplasm. Sperm which are injected into immature eggs at the germinal vesicle stage do not decondense until the germinal vesicle breaks down and even then are unable to form pronuclei (Usui and Yanagimachi, 1976; Thadani, 1979). Conversely, unfertilized mature mammalian oocytes will cause premature chromosome condensation when fused with interphase blastomeres (Balakier, 1979). Thus, the capacity of the oocyte to support pronucleus formation develops towards the end of its meiotic maturation. Pronucleus formation is also dependent on egg activation. Mechanical stimulation of the oocyte plasma membrane or cytoplasm by the micropipette induces egg activation (Uehara and Yanagimachi, 1976). Therefore, injected sperm are deposited into an activated environment and respond as if the egg had been activated by the sperm during the course of normal fertilization.

Induction of the sperm acrosome reaction prior to subzonal insemination has led to higher rates of fertilization in the mouse (Yamada *et al.*, 1988) and increased human sperm penetration of hamster (Mortimer *et al.*, 1986; Lassalle and Testart, 1988) and human (Laws-King *et al.*, 1987) oocytes. Fertilization also occurs in the absence of acrosome reaction inducers (Ng *et al.*, 1988; Yang *et al.*, 1990), although normal physiological capacitation and partial acrosome reaction is likely to have occurred during the preparation of spermatozoa for injection. Pronucleus formation, however, does occur after direct injection of uncapacitated sperm heads (Thadani, 1980) and thus it may be argued that sperm capacitation and the acrosome reaction are relevant only to zona pellucida penetration and sperm–egg fusion and not to pronucleus formation.

The demonstration by Aitken *et al.* (1983) that spermatozoa from men with immotile cilia (Kartagener's) syndrome could fuse with hamster eggs when injected under the zona pellucida and that normal homospecific fertilization could occur after subzonal insertion of spermatozoa into human oocytes (Laws-King *et al.*, 1987; Lazendorf *et al.*, 1988) had exciting ramifications for the alleviation of infertility in man. Shortly thereafter, the first human pregnancy (Ng *et al.*, 1988) and live birth (Fishel *et al.*, 1990) was reported as a result of subzonal insertion of multiple sperm from subfertile men. In addition, microsurgical injection may also be applied to facilitate sperm–egg interaction where there are apparent structural defects in the zona pellucida, i.e. non-functional sperm receptors or zona matrix components which inhibit the passage of sperm.

Modifications of the technique have also been used as alternatives to subzonal insertion. The procedure of zona drilling developed in a mouse model (J.W. Gordon and Talansky, 1986) involves the exposure of a small section of the zona pellucida to a minute volume of acid Tyrode's solution slowly delivered from a microneedle until the zona is breached. Subsequent *in vitro* insemination allows sperm to utilize the artificially created gap (Conover and Gwatkin, 1988; Talansky *et al.*, 1991) and results in increased rates of fertilization in the mouse. Zona drilling has been applied clinically, but the acid Tyrode's solution was found to interfere with normal embryonic development (J.W. Gordon *et al.*, 1988). A mechanical version of the technique, partial zona dissection, which employs a microneedle to form a slit in the

zona pellucida, has, however, been a successfully applied microfertilization technique in the human with normal births resulting (Cohen *et al.*, 1989; Malter and Cohen, 1989). Several other variations on the zona drilling procedure have been attempted, including zona cutting and tearing (Depypere *et al.*, 1988; Odawara and Lopata, 1989) and the novel use of lasers (Godke *et al.*, 1990; Tadir *et al.*, 1991).

2.4.6 PRODUCTION OF CHIMAERAS

Mouse eight-cell eggs were first united in pairs by Tarkowski (1961, 1963), who removed the zona pellucida by drawing the egg into a pipette a bit narrower than the diameter of the egg, and by Mintz (1962, 1964), who digested the zona with the enzyme pronase. The resulting chimaeras revealed random intermingling of cells from the two sources and, on transfer to a recipient, generally developed to birth and functional adulthood. From early days, the need for appropriate cell markers was appreciated. Pigmentation, especially when it was expressed in the iris, was an early favourite; other markers have included enzyme variants (β-glucuronidase, glucose phosphate isomerase, isocitrate dehydrogenase), chromosome variations (T6 translocation in which the homozygote has two small 'marker' chromosomes, differing amounts of pericentric Giemsa-stainable heterochromatin, and for XX/XY chimaeras the presence of the Y chromosome), immunological features (cell-specific antigens detected by haemagglutinin tests or immunofluorescent methods, and skin grafting) and isotope labelling of one component of the chimaera (see McLaren, 1976b, 1981, for full discussion). Use of these methods clearly showed the lack of any pattern in the distribution of the cells from the two sources, unless contrived experimentally, as in the work of Hillman *et al.* (1972) in their investigation of cell determination during blastocyst development (discussed earlier in section 2.2.4). As expected, some chimaeras grew up as hermaphrodites, but most often the male cells assumed dominance and determined the functional sex. More than two cleaving eggs, from the two-cell stage to the late morula, can be induced to form a chimaera, and as many as 16 have been found capable of organizing *in vitro* into a single giant blastocyst of normal proportions.

The work described so far involved the mouse as an experimental animal, but Mayer and Fritz (1974) used the rat, and Moustafa (1974) the rabbit. Other investigators have explored the potential for development of interspecific chimaeras by pairing the zona-free cleaving eggs of mouse and rat (Mulnard, 1973; Stern, 1973; Zeilmaker, 1973; Tachi and Tachi, 1980b) or of mouse and bank vole (Mystkowska, 1975). Well formed expanded rat-mouse blastocysts were obtained, the cells of both species contributing to both SCM and trophectoderm, but implantation after transfer was not observed.

Willadsen and Fehilly (1983), working with sheep eggs, also employed the 'aggregation technique' (as initiated by Tarkowski and by Mintz) to improve the chances of survival of separated blastomeres. Thus, when each of the blastomeres from an eight-cell egg was placed in an empty zona pellucida along with a blastomere from another eight-cell egg, the chances of survival and development were much improved. In one experiment, the blastomeres from an eight-cell Finn–Jacob egg were paired up with blastomeres from an eight-cell Suffolk egg, and five lambs were subsequently born (Figure 2.7).

These were all of the Finn–Jacob phenotype, and this result was attributed to the Finn–Jacob egg being developmentally somewhat in advance of the Suffolk egg. By contrast, when single blastomeres from two two-cell eggs were paired up, the resulting lambs were regularly visibly chimaeric. More recently, interspecific bovine chimaeras have

Figure 2.7 Quintuplet lambs, each arising from a blastomere from an eight-cell Suffolk egg. (Photo by courtesy of S.M. Willadsen.)

been produced by Munro *et al.* (1986) (Figure 2.8).

Composite proembryos can also be constructed by Gardner's method (1968, 1971, 1978, 1982) and are distinguished as 'injection chimaeras'. This procedure involves the introduction of a whole or parts of an inner cell mass from one blastocyst into the cavity of another, the inner cell mass of which may have been partially or wholly removed. Gardner generally used the mouse as experimental animal, but occasionally also the rat (Gardner and Johnson, 1973; Gardner, 1975) and the rabbit (Gardner and Munro, 1974); and other workers have also used the sheep (Tucker *et al.*, 1974, 1978). As with aggregation chimaeras, the introduced cells mingle with those of the other proembryo (though only with the inner cell mass cells in this case), with no detectable pattern in their distribution. The recognizable contribution to the eventual adult animal varies a good deal – one introduced cell sometimes becomes the forerunner of half the final product, as judged by physical characteristics. (Chimaeras can also be produced by aggregating embryonal carcinoma or teratocarcinoma cells with cleaving eggs, or by injecting them into blastocysts – this is discussed later under 'Teratocarcinomas' in section 2.4.8.)

The rare spontaneous chimaeras recorded in clinical studies on the human subject are thought to arise by eggs adhering to each other during cleavage. They should be dis-

tinguished from 'mosaics', which can be very similar in physical characters; mosaics are generally caused by some chromosomal aberration during development, such as loss of a chromosome in mitosis, non-disjunction, somatic mutation or somatic crossing over. (The normal female mammal is, of course, a mosaic with cells differing through X chromosome inactivation.) By definition, mosaics necessarily derive from the fertilization of an egg by a single spermatozoon; if double fertilization occurs (of oocyte and polar body separately), or if cleaving eggs unite, the result is a chimaera.

The study of experimental chimaeras can yield useful information on a variety of developmental problems. One of the first to be investigated was the long-debated origin of skeletal muscle fibres (cells), which contain a central bundle of contractile elements as well as numerous nuclei. In culture *in vitro*, similar cells are formed by fusion of several precursor cells, rather than through multiple karyokinesis without cytokinesis. That this was also the manner of development *in vivo* was demonstrated by Mintz and Baker (1967), who aggregated pairs of mouse morulae that were each homozygous for an electrophoretically different form of the enzyme isocitrate dehydrogenase, and found that in the resulting mice skeletal muscle tissues alone displayed an intermediate form of the enzyme (otherwise found only in F1 hybrids) in addition to the two original forms.

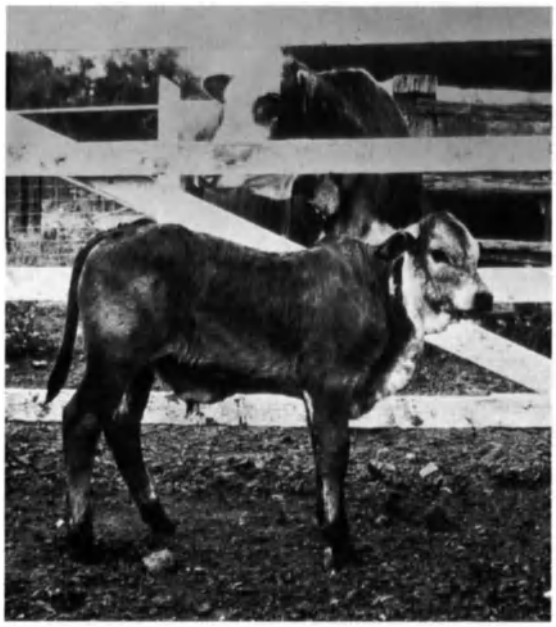

Figure 2.8 Chimaeric calf arising from half a morula from a Brahman cow, aggregated with half a morula from a Hereford–Shorthorn hybrid cow. (Photo by courtesy of R.K. Munro, T.J. Williams and J.N. Shelton, Division of Tropical Animal Science, CSIRO, Rockhampton, Queensland, Australia.)

As mentioned under 'Induction of parthenogenesis' (section 2.4.4), chimaeras have been used to test the viability of the cells of parthenogenones: fusion products of fertilized cleaving eggs and parthenogenones have been found to develop through to fertile adulthood, with the parthenogenetic cells contributing to the formation of fertile gametes (Stevens *et al.*, 1977; Surani *et al.*, 1977; Stevens, 1978). Other examples of such 'rescue' also exist. In T^{fm} male mice, spermatogenesis is arrested in meiosis, but chimaeras formed between XY cleaving eggs and those with $X^{Tfm}Y$ yield adults with normal spermatogenesis, for they sire both XY and $X^{Tfm}Y$ males. The sterility of T^{fm} males is thought to be due to supporting cells (e.g. Sertoli cells) failing to respond to androgen (Lyon *et al.*, 1975). For further details, see McLaren (1981).

Cross-insemination between *Mus musculus* and *M. caroli* rarely yields living young, but when chimaeras were made by injecting ICMs from *M. caroli* into *M. musculus* blastocysts, and transferred to *M. musculus* recipients, the majority developed to adulthood and showed contributions from both sources (Rossant and Frels, 1980, 1981).

Several attempts were made to produce intergeneric aggregation chimaeras between rat and mouse, but development went no further than the blastocyst stage (Mulnard, 1973; Stern, 1973; Zeilmaker, 1973); the injec-

Figure 2.9 A sheep–goat chimaera arising from aggregated four-cell eggs, one from each species. (Photo by courtesy of S.M. Willadsen.)

tion chimaeras of Gardner and Johnson (1973) and Gardner (1975) fared somewhat better, developing to the 33-somite stage. The most advanced stages reported so far are those obtained with sheep–goat aggregation chimaeras, some of which survived to adulthood (Fehilly *et al.*, 1984) (Figure 2.9).

2.4.7 DISAGGREGATION, REAGGREGATION AND SECTION OF PROEMBRYOS

Some of the earliest attempts to investigate the developmental potential of separated blastomeres were those of Nicholas and Hall (1942) and Nicholas (1947) in the rat, but a more definitive phase of research began with Seidel's (1952) success in obtaining development to birth of two-cell rabbit eggs in which one blastomere had been destroyed by needle puncture; four-cell eggs with three blastomeres destroyed developed to somite-stage embryos. Then Tarkowski (1959) separated blastomeres of two- and four-cell mouse eggs and found that cleavage would often continue after transfer to recipients; in one test, six normal young were born (see also Rossant, 1976, 1986). Later, Moore *et al.* (1968) isolated blastomeres from early-cleaving rabbit eggs and found it necessary to replace these in their own zona pellucidas if cleavage were to proceed normally. By this means, full development to birth was obtained at frequencies of 38% for blastomeres from two-cell eggs, 19% for those from four-cell and 11% from eight-cell. In the pig, the survival rate to the blastocyst stage was 35% for single blastomeres from both four- and six-cell eggs (Moore *et al.*, 1969).

Further technical improvements were introduced by Willadsen (1979), who worked with sheep, cow and horse eggs. After transfer of isolated blastomeres to new zonas, he embedded them in small agar cylinders which were lodged in a recipient sheep until blastocyst expansion had begun. The blastocysts were then released and placed in the uterus of a second recipient of the appropriate species (sheep, cow or horse) in which implantation could occur. By these means it proved possible to obtain normal monozygotic twins. Allen and Pashen (1984) produced twin foals by similar means (Figure 2.10).

The blastomeres of later stages were found increasingly less likely to survive the rigours of transfer, presumably because of their progressively smaller nuclear and cytoplasmic volumes; accordingly, several blastomeres were transferred together, and full development was frequently found to be obtainable. When pairs of blastomeres from four-cell eggs, or quartets from eight-cell eggs, were handled together, the probability of normal birth was 70% or better. When a quarter of the blastomere population was used, namely single cells from four-cell eggs or pairs of cells

from eight-cell eggs, the success rate was about 50% or better (Figure 2.11).

Transferring single cells from eight-cell eggs yielded about 10% success. For further details, see Willadsen (1980, 1981), Willadsen and Fehilly (1983) and Fehilly and Willadsen (1986). (The technique of combining blastomeres from different donors was discussed in the previous section on 'Production of chimaeras'.)

Success in obtaining full development with only one or two blastomeres from two-, four- or eight-cell eggs means, of course, that the 'spare' blastomeres could be used for special studies, such as sex determination, karyotyping for chromosomal anomalies, determination of enzyme activity or identification of enzyme variants (see McLaren, 1985). Hardy *et al.* (1990) found that removal of one or two blastomeres from human eight-cell embryos, while reducing the numbers of cells in ICM and trophectoderm at the blastocyst stage, did not affect pre-implantation development. To overcome the handicap of small cell number for analytical work, blastocysts have been cultivated *in vitro* to yield embryonic stem cell lines (Axelrod, 1984); alternatively, growing EK cells *in vitro* as described by Evans and Kaufman (1981) could provide ample material for analysis (as discussed further in the next two sections). Blastocyst biopsy in the rabbit was demonstrated by Gardner and Edwards (1968), and could be applied to other large blastocysts such as those of sheep, cattle and horses. Trophectoderm biopsy of human blastocysts can provide sufficient material for genetic diagnosis without impairing hatching potential (Dokras *et al.*, 1990). With the aid of high-performance liquid chromatography, Leese *et al.* (1991) were able to assay the

Figure 2.10 Twin foals produced by separating the blastomeres of a two-cell egg. (Photo by courtesy of W.R. Allen.)

Figure 2.11 Identical triplet calves, each deriving from two blastomeres of the same eight-cell egg (the product of the fourth pair failed to survive the birth). (Photo by courtesy of S.M. Willadsen.)

enzyme HPRT in single cleavage-stage human blastomeres.

Monozygotic cattle twins have been obtained by transferring bisected morulas to recipients (Ozil *et al.*, 1982; Lambeth *et al.*, 1983; Baker *et al.*, 1984; Voekel *et al.*, 1984). Baker *et al.* (1984) obtained 79% of pregnancies with entire embryos and 68% with 'split' (bisected) specimens, the difference being insignificant, and Voekel *et al.* (1984) 42.6% with both. Tsunoda *et al.* (1985b) have reported the birth of twins in Japanese native goats following this procedure.

2.4.8 TERATOCARCINOMAS

These tumours can develop spontaneously from diploid spermatogonia in the testis or from oocytes after the first meiotic division (particularly in the LT/Sv mouse strain), but in the present context attention will be restricted essentially to teratocarcinomas arising from transplanted mouse embryos (a field reviewed by Stevens, 1981). Some of the earliest work involved the initiation of teratomas by inserting cleaving eggs or six-day egg cylinders from mice of the 129 strain under the testis capsule; after passing through some normal stages of development, the cells became disorganized and growth continued as a teratoma. Induced in this way, teratomas are, therefore, derived from somatic cells, in contrast to the germ cell origin of the spontaneous tumours. A minor proportion of the growths become teratocarcinomas, consisting of undifferentiated em-

bryonal carcinomic cells which can be maintained by reinsertion. The cells have been shown to be pluripotent or even totipotent, but after several transfers some lose their ability to produce some or any kind of differentiated cell.

Disaggregated teratocarcinomas injected into the peritoneal cavity can give rise to retransplantable ascites tumours, the ascitic fluid suspending many 'embryoid bodies'. These resemble the egg cylinder stage of development, with organized layers of embryonic ectoderm and endoderm (Stevens, 1958, 1964; Pierce and Dixon, 1959), or else a core of EC cells surrounded by a rind of cells resembling endoderm.

EC cells from a mouse of a black strain, when injected into the cavity of blastocysts from a white strain, became integrated with the inner cell mass cells and later contributed to parts of normal adult mice, as shown by the pigmented patches of fur (Brinster, 1974).

These two lines of enquiry were drawn together when Mintz and Illmensee (1975) took embryoid bodies from mice with ascites tumours that had been passaged between recipients about 200 times, dissected off the rind and disaggregated the core cells (Figure 2.12).

These cells were injected into the cavity of blastocysts which later developed into mice exhibiting coat colour mosaicism, testifying to a contribution from the embryoid bodies. More than that, when these mice were mated with untreated animals, some of the resulting young had coat colours indicating that the core cells from embryoid bodies had given rise to normal functioning gametes. The fact that the properties shown by the embryoid bodies could be matched by those of EC cells maintained in culture was shown by Papaioannou *et al.* (1978).

A few years later, Evans and Kaufman (1981) found that, if experimentally delayed mouse blastocysts were held in culture, they became attached to the dish and trophoblast cells grew out, the inner cell mass differentiating into an egg cylinder-like structure (Figure 2.13).

This could be removed, disaggregated and grown in culture over a feeder layer of inactivated fibroblasts. Soon, colonies of EC cells could be discerned and subcultured; they were found to have a normal karyotype and often displayed a Y chromosome, both features distinguishing them from corresponding cells produced by other investigators. These cells were accordingly identified as EK cells; they behaved in much the same way as EC cells, giving rise to teratocarcinomas on subcutaneous injection. When cultured without feeder cells, they formed embryoid bodies. When introduced into the cavity of blastocysts, the resulting adult mice showed visible evidence of the chimaeric origin (Figure 2.14), and mating studies revealed that several animals were functional germ cell chimaeras (Bradley *et al.*, 1984).

More recently, EK cells have come to be known as ES (embryonic stem) cells (see Robertson, 1987, and Jackson, 1989). Notarianni *et al.* (1990) have described the establishment in culture of cell lines from pig blastocysts that resemble mouse ES lines.

In the meantime, Fujii and Martin (1980) and C.L. Stewart (1982) reported that viable chimaeras consisting of normal cells and carcinoma cells could be produced not only by injection but also by aggregation of the carcinoma cells with those of eight-cell embryos. Subsequent development, however, was often abnormal, the degree of anomaly correlating with the proportion of carcinoma cells composing the fetus – when the proportion was large, the fetus was unlikely to reach term. The possibility existed that the ES cell lines tested (NG-2, PSA-1 and LT1-2D) failed because of chromosomal abnormalities. Rossant and McBurney (1982), however, found that major developmental errors were often associated with carcinoma cells that had normal karyotypes.

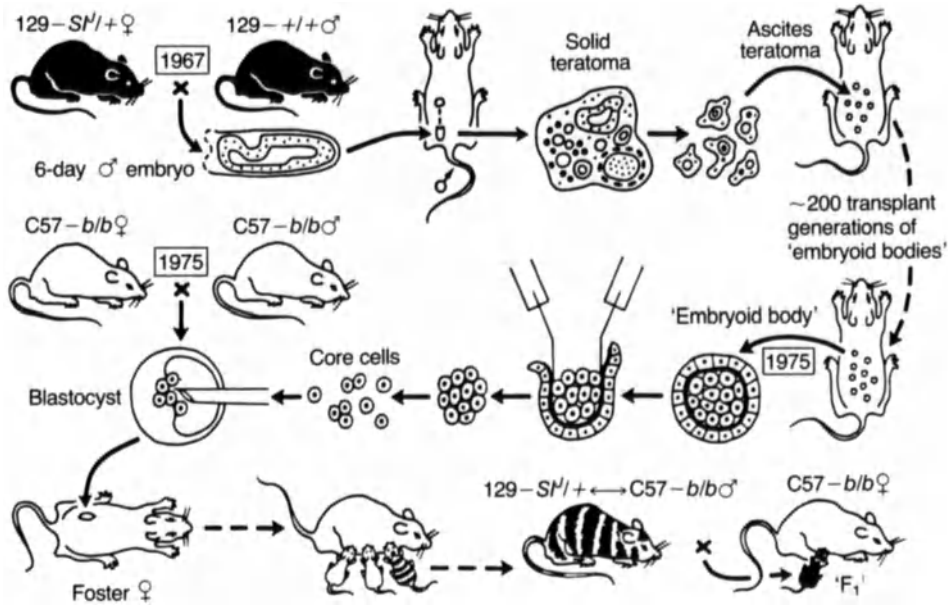

Figure 2.12 The remarkable experiment of Mintz and Illmensee (1975). (See section 2.4.8 for details.)

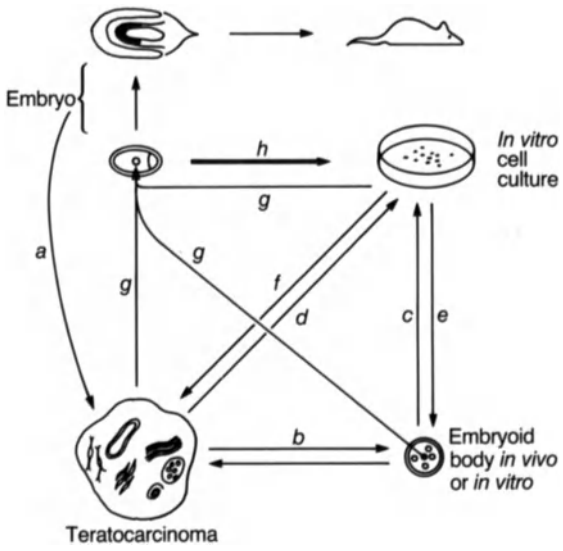

Figure 2.13 The potentials of mouse inner cell mass cells when cultured under different conditions. The arrow marked 'h' represents the accomplishment of Evans and Kaufman (1981) in obtaining clones of pluripotential 'EK' cells of normal karyotype directly from blastocysts.

2.4.9 RECOMBINANT DNA RESEARCH

Probably the first indication that a subcellular substance could transmit a heritable character between individuals came with the observations of Griffith (1928) on the exchange of colony characters between S and R strains of *Streptococcus pneumoniae*; the active agent became known as the 'transforming principle'. Some years later, Avery *et al.* (1944) demonstrated that this was, in fact, DNA. So our understanding of transformation as a means of gene shift between individuals preceded recognition of 'conjugation' – the normal mode of sexual reproduction in bacteria, seen first in *Escherichia coli*, whereby lengths of chromosome are passed from one cell to another (Lederberg and Tatum, 1946). Shortly afterwards, Zinder and Lederberg (1952) observed 'transduction' of genes between strains of *Salmonella*, effected by a temperate bacteriophage. In this process, a fragment of bacterial chromosome becomes incorporated into the virus particle and is carried to the next host cell.

Exploitation of viruses for the transfer of genetic information between mammalian cells would appear to flow logically from these observations, yet it was about 20 years before F.L. Graham and Van Der Eb (1973), interested in the infectivity of viral DNA, obtained transformation of cultured rat cells by the DNA of adenovirus. A year later, Jaenisch and Mintz (1974) announced that, when intact DNA from SV40 (a simian retrovirus) was injected into the cavity of mouse blastocysts, viral DNA sequences were later found incorporated into the adult mouse somatic tissues. A much higher success rate was obtained when J.W. Gordon *et al.* (1980) inserted into the plasmid pBR322 the herpes simplex thymidine kinase gene together with the SV40 virus origin of replication and injected the product into pronuclei of mouse eggs, which were immediately placed in recipient oviducts. Among the mice that developed to birth, some exhibited sequences in somatic tissues that were homologous with the injected plasmid, but there was no evidence of incorporation into the germ line. This step was achieved by Jähner and Jaenisch (1980) by viral infection of early mouse proembryos, and also by Costantini and Lacy (1981) by injection of a cloned rabbit DNA fragment containing the adult β-globin gene into the pronucleus in mouse eggs; in both instances, transmission to subsequent progeny was demonstrated. T.A. Stewart *et al.* (1982) were able to transfer human β-globin gene sequences to mice by pronuclear injection, and there was evidence of transmission to progeny by one animal.

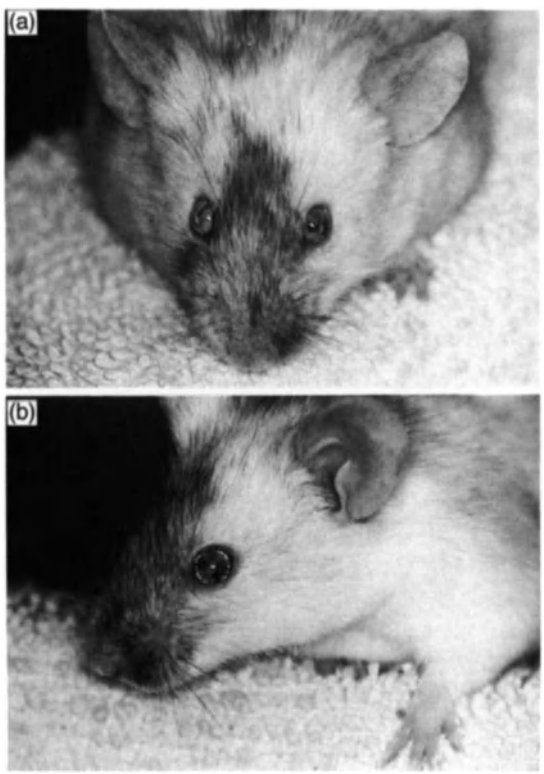

Figure 2.14 Chimaerism shown by the pigmentation of fur and iris in an adult mouse, which developed from a 'white' blastocyst into which EK cells from a black strain had been injected. (Figure 5.7 from Kaufman, 1983.)

But the striking advance in this phase of research was the production of larger-than-normal mice, in some instances nearly twice the body weight of controls and maintaining approximately a fourfold elevation of circulating growth hormone levels (Palmiter *et al.*, 1982). This was achieved by injecting into pronuclei of mouse eggs a DNA fragment containing the structural gene of rat growth hormone fused to the promoter of the mouse metallothionein I (MT-I) gene. Administration of zinc to the resulting animals stimulated the MT-I gene, which in turn evoked expression of the growth hormone gene. The possibility that the introduced rat growth hormone genes were integrated into a single chromosome of the mouse genome was supported by the pattern of inheritance through two successive generations (Palmiter and Brinster, 1984). The transfer of human growth hormone genes to mice by a similar means also resulted in growth promotion (Palmiter *et al.*, 1983), and similar results were obtained by transfer of the human gene for growth hormone-releasing hormone (Hammer *et al.*, 1985). The development of

gene transfer methods in cattle is described by Roschlau *et al.* (1989). More recently, Pursel *et al.* (1990) have reported functional integration and inheritance of growth-related genes introduced into pigs, and Rexroad *et al.* (1990) have commented on the need for better control of inserted genes in ruminants. Other genes are also worthy of attention (Ward *et al.*, 1984; Land and Wilmut, 1987; First, 1990).

Prospects for the advantageous application of recombinant DNA technology to human problems depend firstly on reliable diagnosis of genetic diseases (see Gosden and Gosden, 1985; Weatherall, 1985; and Akhurst, 1986, for reviews). Sometimes, diagnosis can be made by relatively simple biochemical means; for example, determination of the normality or otherwise of the gene for hypoxanthine phosphoribosyl transferase can be based on analyses for the presence or absence of the enzyme in single blastomeres from 8-cell mouse eggs.

More often, however, dependence must be placed on diagnosis much later, as in the examination of DNA in amniotic fluid cells collected usually in the sixteenth week of gestation, though adequate material can commonly be obtained by chorionic villus biopsy in the eighth to twelfth week of gestation (Rhine and Milunsky, 1979; Williamson *et al.*, 1981; Maxwell *et al.*, 1985; Brambati, 1986; Brambati *et al.*, 1986; and see also abstracts in Human Reproduction, 1, pp. Al 1-A13, A29-A30, 1986). For direct chromosome studies, at least 5mg of villous tissue is necessary for the preparation of useful karyotypes (Dalpra *et al.*, 1986). Satisfactory genomic analysis, however, can be made on 10 g of tissue, based on 'restriction endonuclease mapping'. Endonucleases cleave the DNA molecule at different highly specific points, and the normal length of the fragments and their localizations relative to particular genes are known, so that it is possible to detect mutations that produce new sites of enzyme action, or remove sites, or cause the addition or loss of one or more genes. This technique has been used successfully for the early recognition of sickle cell anaemia, some forms of β-thalassaemia and phenylketonuria, and a marker has also been discovered for Huntington's chorea, but there are at present difficulties in the wider application.

That sex determination at an early stage is of undeniable value has led to important advances in the development of DNA probes for the detection of X- and Y-specific DNA sequences in the pre-implantation embryo. The sensitivity of DNA analyses permits genetic diagnoses to be made utilizing a single cell. At present, it is possible to determine the sex of an embryo by analysing a single extracted blastomere using either *in situ* hybridization or the polymerase chain reaction.

In situ hybridization is a technique that allows direct visualization of specific DNA sequences. Initially, the procedure utilized radiolabelled probes coupled with autoradiography, and was used to detect Y chromosome-specific repeated nucleotide sequences in the human male embryo (Jones *et al.*, 1987) and in domestic species (Leonard *et al.*, 1987; Matthews *et al.*, 1987). More recently, rapid non-isotopic methods have been used for the detection of both X- and Y-chromosome sequences in human pre-implantation embryos (Penketh *et al.*, 1989; Griffin *et al.*, 1992). Since signals are visualized directly under the microsope, localization of the signal is more precise. Multiple signals using different reporter molecules such as fluorescein isothiocyanate (FITC) and rhodamine give different colour signals. Using different fluorochromes, it is possible to detect a number of autosomes as well as the sex chromosomes simultaneously, which opens up the possibility of using this technique for the detection of inherited disorders.

The polymerase chain reaction is a method which amplifies specific sequences of DNA for analysis. The sequence may be specific to a sex chromosome or may include a site of

deletion or mutation. The technique has been applied successfully for the screening of human pre-implantation embryos prior to transfer by determining the gender using Y-specific DNA amplification (Handyside *et al.*, 1990) and by the diagnosis of single-gene defects such as cystic fibrosis (Hardy and Handyside, 1992).

Although these techniques open up new frontiers in genetic diagnosis, the technology is currently limited. Analysis of a single blastomere from an eight-cell embryo may not detect mosaicism or genetic modification at a later stage, and the methodology itself can have problems such as loss or inactivation of DNA, amplification failure and contamination.

The problems of gene therapy are even more forbidding. The haploid human genome is considered to contain 3×10^9 base pairs, and some 50 000 to 100 000 structural genes. Many human genes have been isolated, along with their flanking regions, where regulatory sequences are likely to be found, and gene insertion into foreign cells has been widely achieved – for example, the recent transfer, with the aid of a mouse retrovirus, of a human gene for HPRT from a normal cell to a deficient cell, with transfer of appropriate function – but here there is great lack of tissue specificity. Some functions, such as the production of growth hormone, can evidently be performed by a wide range of cells with physiologically consistent consequences, but other functions, such as haemoglobin production, must be the prerogative of particular cells at particular stages of development. The problem is how to insert genes into selected target cells, and this is discussed by Weatherall (1985), with special reference to the haemoglobinopathies. Leads for future research may be derived from study of the so-called 'hotspots' or preferred sites for gene insertion, which could represent sites of DNAase hypersensitivity (Storb *et al.*, 1979). More recently, Smithies *et al.* (1985) described the insertion of DNA sequences into the human β-globin locus by homologous recombination, a procedure that could be applicable to the treatment of conditions such as thalassaemia and sickle cell anaemia.

Another and highly productive line of research in laboratory animals concerns the use of retroviruses and other foreign DNA forms to identify genes important in embryonic development. This has been exploited by Jähner and Jaenisch (1980) and more recently by Jaenisch *et al.* (1983), Schnieke *et al.* (1983), Wagner *et al.* (1983) and Jaenisch (1984). The introduced material either enhances the host gene transcription by the action of a strong promoter, or more commonly disrupts the host gene, both actions usually leading to a lethal mutation. The foreign DNA is generally capable of becoming inserted at many points in the host genome, apparently at random, so that the function of virtually any gene can be affected. The consequences of these changes can be studied by genetic analysis, recognition of significant effects depending on the inviability of homozygotes.

The following books are suggested as further reading: Connor and Ferguson-Smith (1984, 1987), Emery (1984), Glover (1984), Nossal (1985), Warr (1984); Jackson, (1989); Hansel and Weir (1990).

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REFERENCES

Abramczuk, J.W. and Lopata, A. (1990) Resistance of human follicular oocytes to parthenogenetic activation: DNA distribution and content in oocytes maintained *in vitro*. *Human Reproduction*, **5**, 578–581.

Adams, C.E. (1957) An attempt to cross the domestic rabbit (*Oryctolagus cuniculus*) and hare (*Lepus europaeus*). *Nature* **180**, 853.

Adams, C.E. (1975) Bibliography on recovery and transfer of mammalian eggs. *Bibliography of Reproduction*, Vol. 89, Reproduction Research Information Service, Cambridge.

Adams, C.E. (1977) Bibliography of recovery and transfer of mammalian eggs. *Bibliography of Reproduction*, Vol. 101, Reproduction Research Information Service, Cambridge.

Adams, C.E. (ed.) (1982) *Mammalian Egg Transfer*, CRC Press, Boca Raton, Florida.

Adams, C.E. and Abbott, M. (1971) Bibliography on recovery and transfer of mammalian eggs and ovarian transplantation. *Bibliography of Reproduction*, Vol. 45, Reproduction Research Information Service, Cambridge.

Adamson, E.D. and Gardner, R.L. (1979) Control of early development in *Reproduction*, (ed. R.V. Short), British Medical Bulletin, **35**, pp. 113–119.

Ahuja, K.K., Smith, W., Tucker, M., and Craft, I. (1985) Successful pregnancies from the transfer of pronuncleate embryos in an outpatient *in vitro* fertilization programme. *Fertility and Sterility*, **44**, 181–184.

Aitken, R.J. (1977) Embryonic diapause in *Development in Mammals*, Vol. 1 (ed. M.H. Johnson), North Holland Publishing Company, Amsterdam.

Aitken, R.J., Ross, A. and Lees, M. (1983) Analysis of sperm function in Kartagener's Syndrome. *Fertility and Sterility*, **40**, 696–698.

Akhurst, R.J. (1986) The use of gene probes in studying human reproduction and embryology. *Human Reproduction*, **1**, 213–218.

Allen, W.R. and Pashen, R.O. (1984) Production of monozygotic (identical) horse twins by embryo micromanipulation. *Journal of Reproduction and Fertility*, **71**, 607–613.

Amoroso, E.C. (1952) Placentation, in *Marshall's Physiology of Reproduction*, 3rd edn, (ed. A.S. Parkes), Longman, Green and Co. London.

Amoroso, E.C., Griffiths, W.F.B. and Hamilton, W.J. (1942) The early development of the goat (*Capra hircus*). *Journal of Anatomy*, **76**, 377–406.

Anderegg, C. and Markert, C.L. (1986) Successful rescue of microsurgically produced homozygou uniparental mouse embryos via production of aggregate chimeras. *Proceedings of the National Academy of Sciences of the USA*, **83**, 6509–6513.

Anon. (1985) A multinational case–control study of ectopic pregnancy. *Clinical Reproduction and Fertility*, **3**, 131–143.

Asch, R.H., Ellsworth, L.R. and Balmaceda, J.P. (1984) Pregnancy following translaparoscopic intrafallopian transfer (GIFT). *Lancet*, **ii**, 1034.

Ashwood-Smith, M.J. (1986) The cryopreservation of human embryos. *Human Reproduction*, **1**, 319–332.

Austin, C.R. (1949) The fragmentation of eggs following induced ovulation in immature rats. *Journal of Endocrinology*, **6**, 104–110.

Austin, C.R. (1956) Activation of eggs by hypothermia in rats and hamsters. *Journal of Experimental Biology*, **33**, 338–347.

Austin, C.R. (1961) *The Mammalian Egg*, Blackwell Scientific Publications, Oxford.

Austin, C.R. (1973) Embryo transfer and sensitivity to teratogenesis. *Nature*, **244**, 333–334.

Austin, C.R. (1987) Are sperms really necessary? in *New Horizons in Sperm Cell Research*, (ed. H. Mohri), Japan Scientific Societies Press, Tokyo, Gordon and Breach Science Publishers, New York, pp. xxi–xxix.

Austin, C.R. and Walton, A. (1960) Fertilization, in *Marshall's Physiology of Reproduction*, 3rd edn, Vol. 1, pt 2, (ed. A.S. Parkes), Longman, Green and Co, London, pp. 310–416.

Avery, O.T., McLeod, C.M. and McCarty, M. (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types – induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus Type III. *Journal of Experimental Medicine*, **79**, 137–158.

Axelrod, H.R. (1984) Embryonic stem cell lines derived from blastocysts by a simplified technique. *Developmental Biology*, **101**, 225–228.

von Baer, K.E. (1827) *De Ovi Mammalium et Hominis Genesi*, Lipsiae.

von Baer, K.E. (1837) *Ueber Entwickelungsgeschichte der Thiere*, Beobachtung und Reflexion, Th. 2 Konigsberg.

Baker, R.D., Eberhard, B.K., Leffel, R.E., Rohde, R.F. and Henschen, T.J. (1984) Pregnancy rates following surgical transfer of bovine demi-embryos. *10th International Congress on Animal Reproduction and Artificial Insemination*, Urbana-Champaign, USA, June 10–14.

Balakier, H. (1979) Interspecific heterokaryons between oocytes and blastomeres of the mouse and bank vole. *Journal of Experimental Zoology*, 323–329.

Balmaceda, J.P., Borrero, C., Castaldi, C., Ord, T., Remohi, J. and Asch, R.H. (1988) Tubal embryo transfer as a treatment for infertility due to male factor. *Fertility and Sterility*, **50**, 476–479.

Barry, M. (1838) Researches in embryology – first series. *Philosophical Transactions of the Royal Society of London*, pt 1, 301–342.

Barry, M. (1839) Researches in embryology – second series. *Philosophical Transactions of the Royal Society of London*, pt 2, 307–380.

Barton, S.C., Surani, M.A.H. and Norris, M.C. (1984) Role of paternal and maternal genomes in mouse development. *Nature*, **311**, 374–376.

Barton, S.C., Adams, C.A., Norris, M.L. and Surani, M.A.H. (1985) Development of gynogenetic and parthenogenetic inner cell mass and trophectoderm tissues in reconstituted blastocysts in the mouse. *Journal of Embryology and Experimental Morphology*, **90**, 267–285.

Bauer, O., Van der Ven, H., Diedrich, K., Al-Hasani, S., Krebs, D. and Grenibruch, U. (1990) Preliminary results on transvaginal tubal embryo stage transfer (TV-TEST) without ultrasound guidance. *Human Reproduction*, **5**, 553–556.

Bavister, B.D. (1986) Animal *in vitro* fertilization and embryo development in *Manipulation of Mammalian Development, Developmental Biology*, Vol. 4 (ed. R.B.L. Gwatkin), Plenum Press, New York.

Bavister, B.D., Boatman, D.E., Liebfried, L., Loose, M. and Vernon, M.W. (1983) Fertilization and cleavage of rhesus monkey oocytes *in vitro*. *Biology of Reproduction*, **28**, 983.999.

Bavister, B.D., Boatman, D.E., Collins, K., Dierschke, D.J. and Eisele, S.G. (1984) Birth of rhesus monkey infant after *in vitro* fertilization and non-surgical embryo transfer. *Proceedings of the National Academy of Sciences of the USA*, **81**, 2218–2222.

Beatty, R.A. (1957) *Parthenogenesis and Polyploidy in Mammalian Development*, Cambridge University Press, London.

Beatty, R.A. (1967) Parthenogenesis in vertebrates in *Fertilization*, vol 1, (eds C.B. Metz and A. Monroy), Academic Press, New York, pp. 413–440.

Beier, H.M. (1968) Uteroglobin, a hormone-sensitive endometrial protein involved in blastocyst development. *Biochimica et Biophysica Acta*, **160**, 289–291.

Belling, R., Haaf, H., Mayall, R., Metzler, M. and Beier, H.M. (1985) Oxidative and conjugative metabolism of diethylstilbestrol by rabbit preimplantation embryos. *Developmental Biology*, **109**, 370–374.

Bergstrom, S. (1978) Experimentally delayed implantation in *Methods in Mammalian Reproduction*, (ed. J.C. Daniel Jr.), Academic Press, New York, pp. 419–435.

Betteridge, K.J. (ed.) (1977) *Embryo Transfer in Farm Animals*, Canada Department of Agriculture Monograph 16.

Betteridge, K.J. (1986) Increasing productivity in farm animals in *Reproduction in Mammals, Book No 5, Manipulating Reproduction*, Chapter 1 (eds C.R. Austin and R.V. Short), Cambridge University Press, Cambridge.

Betteridge, K.J. and Mitchell, D. (1974) Direct evidence of retention of unfertilized ova in the oviduct of the mare. *Journal of Reproduction and Fertility*, **39**, 145–148.

Biggers, J.D. and Borland, R.M. (1976) Physiological aspects of growth and development of the preimplantation mammalian embryo. *Annual Review of Physiology*, **38**, 95–119.

Biggers, J.D., Whittingham, D.G. and Donahue, R.P. (1967) The pattern of energy metabolism in the mouse oocyte and zygote. *Proceedings of the National Academy of Sciences of the USA*, **58**, 560–567.

Bindon, B.M. (1971) Systematic study of preimplantation stages of pregnancy in the sheep. *Australian Journal of Biological Science*, **241**, 131–147.

Bischoff, T.L.W. (1842a) *Entwicklungsgeschichte des Kanincheneies*, Braunschweig.

Bischoff, T.L.W. (1842b) *Entwicklungsgeschichte des Menschen und des Saugethiere*, Leipzig.

Bischoff, T.L.L. (1845) *Entwicklungsgeschichte des Hundeseies*, Braunschweig.

Bischoff, T.L.W. (1852) *Entwicklungsgeschichte des Meerschweinschens*, Giessen.

Bischoff, T.L.S. (1854) *Entwicklungsgeschichte des Rehes*, Giessen.

Blandau, R.J. (1949) Observations on the implantation of the guinea pig ovum. *Anatomical Record*, **103**, 19–47.

Blandau, R.J. (1961) Biology of eggs and implantation in *Sex and Internal Secretions*, 3rd edn, Chapter 14 (eds W.C. Young and G.W. Corner), Bailliere, Tindall and Cox, London.

Blandau, R.J. (1973) Gamete transport in the female mammal in *Handbook of Physiology*, Section 7, Endocrinology II, (eds R.O. Greep and E.B. Astwood), American Physiological Society, Washington, pp. 153–163.

Boatman, D.E. (1987) *In vitro* growth of non-human primate pre- and peri-implantation em-

bryos in *The Mammalian Preimplantation Embryo: Regulation of Growth and Differentiation In Vitro*, (ed. B.D. Bavister), Plenum Press, New York.

Boell, E.J. and Nicholas, J.S. (1948) Respiratory metabolism of the mammalian egg. *Journal of Experimental Zoology*, **109**, 267–281.

Bolton, V.N., Oades, P.J. and Johnson, M.H. (1984) The relationship between cleavage, DNA replication, and gene expression in the mouse 2-cell embryo. *Journal of Embryology and Experimental Morphology*, **79**, 139–163.

Bongso, A., Ng., S.C., Fong, C.Y., Anandakumar, C., Marshall, B., Edirisinghe, R. and Ratnam, S. (1992) Improved pregnancy rate after transfer of embryos grown in fallopian tubal cell coculture. *Fertility and Sterility*, **58**, 569–574.

Borland, R.M., Biggers, J.D. and Lechine, C.P. (1967) Regulation of rabbit blastocele potassium by Na^+-K^+ ATPase on the juxtacoelic surface of the trophoblast cells. *Society for the Study of Reproduction*, abstract.

Boshier, D.P. (1969) A histological and histochemical examination of implantation and early placentome formation in sheep. *Journal of Reproduction and Fertility*, **19**, 51–61.

Boyd, J.D. and Hamilton, W.J. (1952) Cleavage, early development and implantation of the egg in *Marshall's Physiology of Reproduction*, 3rd edn, Vol. II, Chapter 14, (ed. A.S. Parkes), Longman, Green and Co, London.

Boyd, J.D., Hamilton, W.J. and Hammond Jr., J. (1944) Transuterine ('internal') migration of the ovum in sheep and other animals. *Journal of Anatomy*, **78**, 5–14.

Brackett, B.G., Seidel Jr., G.E. and Seidel, S.M. (1981) *New Technologies in Animal Breeding*, Academic Press, Amsterdam.

Bradley, A., Evans, M., Kaufman, M.H. and Robertson, E. (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature*, **309**, 255–256.

Brambati, B. (1986) Current status of first trimester fetal diagnosis of genetic diseases. *Human Reproduction*, **1**, 333–334.

Brambati, B. and Tului, L. (1990) Preimplantation genetic diagnosis: a new simple uterine washing system. *Human Reproduction*, **5**, 448–450.

Brambati, B., Guercilena, S., Bonachhi, I., Oldrini, A., Lanzani, A. and Piceni, L. (1986) Feotmaternal transfusion after chorionic villus sampling; clinical implications. *Human Reproduction*, **1**, 37–40.

Brambell, F.W.R. and Hemmings, W.A. (1949) The passage into the embryonic yolk-sac cavity of maternal plasma proteins in rabbits. *Journal of Physiology*, **108**, 177–185.

Braude, P., Bolton, V. and Moore, S. (1988) Human gene expression first occurs between the 4- and 8-cell stages of preimplantation development. *Nature*, **332**, 459–461.

Briggs, R. (1977) Genetics of cell type determination, in *Cell Interactions in Differentiation*, (eds M. Karinen-Jääskeläinen, L. Saxén and L. Weiss), Academic Press, London, New York, San Francisco, pp. 23–43.

Briggs, R. and King, T.J. (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proceedings of the Academy of Science*, **38**, 455–463.

Brinster, R.L. (1967a) Carbon dioxide production from glucose by the pre-implantation mouse embryo. *Experimental Cell Research*, **47**, 271–277.

Brinster, R.L. (1967b) Carbon dioxide production from lactate and puruvate by the pre-implantation mouse embryo. *Experimental Cell Research*, **47**, 634–637.

Brinster, R.L. (1972) Developing zygote in *Reproductive Biology*, chap 20, (eds H. Balin and S. Glasser), Excerpta Medica, Amsterdam, pp. 748–775.

Brinster, R.L. (1974) The effect of cells transferred into the mouse blastocyst on subsequent development. *Journal of Experimental Medicine*, **140**, 1049–1056.

Bromhall, J.D. (1975) Nuclear transplantation in the rabbit egg. *Nature*, **258**, 719–722.

Bryden, M.M., Evans, H.E. and Binns, W. (1972) Embryology of the sheep. I. Extraembryonic membranes and the development of body form. *Journal of Morphology*, **138**, 169–185.

Buster, J.E., Bustillo, M., Thornycroft, I.H., Simon, J.A., Boyers, S.P., Marshall, J.R., Louw, J.A., Seed, R.W. and Seed, R.G. (1983) Non-surgical transfer of *in vivo* fertilized donated ova to five infertile women: report of two pregnancies. *Lancet*, **ii**, 223–224.

Bustillo, M., Munabi, A.K. and Schulman, J.D. (1988) Pregnancy after nonsurgical ultrasound-guided gamete intrafallopian transfer. *New England Journal of Medicine*, **319**, 313.

Calarco, P.G. and Szollosi, D.G. (1973) Intracisternal A particles in ova and preimplantation stages of the mouse. *Nature, New Biology*, **243**, 91–93.

Cann, R.L., Stoneking, M. and Wilson, A.C.

(1987) Mitochondrial DNA and human evolution. *Nature*, **325**, 31–36.

Carney, E.W. and Foote, R.H. (1991) Improved development of rabbit one-cell embryos to the hatching blastocyst stage by culture in a defined, protein-free culture medium. *Journal of Reproduction and Fertility*, **91**, 113–123.

Cech, S. and Sedlackova, M. (1983) Ultrastructure and morphometric analysis of preimplantation mouse embryos. *Cell and Tissue Research*, **230**, 661–670.

Chang, M.C. (1947) Normal development of fertilized rabbit ova sorted at low temperature for several days. *Nature*, **159**, 602.

Chang, M.C. (1959) Fertilization of rabbit ova *in vitro*. *Nature*, **184**, 466.

Chang, M.C. (1960) Fertilization of domestic rabbit (*Oryctolagus cuniculus*) ova by cottontail rabbit (*Sylvilagus transitionalis*) sperm. *Journal of Experimental Zoology*, **144**, 1–10.

Chang, M.C. (1965) Artificial insemination of Snowshoe hares (*Lepus americanus*) and the transfer of their fertilized eggs to the rabbit (*Oryctolagus cuniculus*). *Journal of Reproduction and Fertility*, **10**, 447–449.

Chang, M.C. and Adams, E.C. (1962) Fate of rabbit ova fertilized by hare spermatozoa. *XXV Anno della Fondazione dello Instituto Sperimentale Italiano L. Spallanzani*, p. 186.

Chang, M.C. and Hancock, J.L. (1967) Experimental hybridization in *Comparative Aspects of Reproductive Failure*, (ed. K. Benirschke), Springer-Verlag, New York, pp. 206–217.

Chang, M.C. and McDonough, J.L. (1955) An experiment to cross the cottontail and domestic rabbit. *Journal of Heredity*, **45**, 41–44.

Chang, M.C. and Pickworth, S. (1969) Egg transfer in the laboratory animal in *The Mammalian Oviduct* (eds E.S.E. Hafez and R.J. Blandau), University of Chicago Press, pp. 389–405.

Chang, M.C., Marston, J.H. and Hunt, D.M. (1964) Reciprocal fertilization between the domestic rabbit and the Snowshoe hare with special reference to insemination of rabbits with an equal number of hare and rabbit spermatozoa. *Journal of Experimental Zoology*, **155**, 437–445.

Chatot, C.L., Tasca, R.J. and Ziomek, C.A. (1990) Glutamine uptake and utilization by preimplantation mouse embryos in C.Z.B. medium. *Journal of Reproduction and Fertility*, **89**, 335–346.

Chen, C. (1986) Pregnancy after human oocyte cryopreservation. *Lancet*, **i**, 884–886.

Chen, L.T. and Hsu, Y.C. (1982) Development of mouse embryos *in vitro*: preimplantation to limb bud stage. *Science*, **218**, 66–68.

Cholewa, J. and Whitten, W.K. (1970) Development of 2-cell mouse embryos in the absence of a fixed nitrogen source. *Journal of Reproduction and Fertility*, **22**, 553–555.

Cohen, J., Simons, R.F., Fehilly, C.B., Edwards, R.G., Hewitt, J., Rowland, G.F., Steptoe, P.C. and Webster, S.M. (1985) Birth after replacement of hatching blastocyst cryopreserved at expanded blastocyst stage (letter). *Lancet*, **i**, 647.

Cohen, J., Malter, H., Wright, G., Kort, H. Massey, J. and Mitchell, D. (1989) Partial zona dissection of human oocytes where failure of zona pellucida penetration is anticipated. *Human Reproduction*, **4**, 435–442.

Cole, R.J. and Paul, J. (1965) Properties of cultured preimplantation mouse and rabbit embryos, and cell strains derived from them in *Preimplantation Stages of Pregnancy*, (eds G.E.W. Wolstenholme and M. O'Connor), Churchill, London, pp. 82–112.

Confino, E., Friberg, J. and Gleicher, N. (1986) Transcervical balloon tublasty. *Fertility and Sterility*, **46**, 963–966.

Connor, J.M. and Ferguson-Smith, M.A. (1984) *Essential Medical Genetics*, Blackwell Scientific Publications, Oxford.

Conover, J.C. and Gwatkin, R.B.L. (1988) Fertilization of zonadrilled mouse oocytes treated with a monoclonal antibody to the zone glycoprotein, ZP3. *Journal of Experimental Zoology*, **247**, 113–118.

Costantini, F. and Lacy, E. (1981) Introduction of a rabbit β-globin gene into the mouse germ line. *Nature*, **294**, 92–94.

Coutinho, E.M. (1974) Hormonal control of oviductal motility and secretory functions in *Reproductive Physiology*, (Vol. 8 in Physiology, Series One. MTP International Review of Science), (ed. R.O. Greep), Butterworth, London; University Park Press, Baltimore, pp. 133–153.

Craft, I. (1984) *In vitro* fertilization – clinical methodology. *British Journal of Hospital Medicine*, **31**, 90–102.

Crosby, I.M., Gandolfi, F. and Moor, R.M. (1988) Control of protein synthesis during early cleave of sheep embryos. *Journal of Reproduction and Fertility*, **82**, 769–775.

Cross, M.H. (1973) Active sodium and chloride transport across the rabbit blastocoele wall. *Biology of Reproduction*, **8**, 566–575.

Cross, M.H. and Brinster, R.L. (1969) Trans membrane potential of the rabbit blastocyst trophoblast. *Experimental Cell Research*, **58**, 125–127.

Croxatto, H.B., Fuentealba, B., Diaz, S., Pastene, L. and Tatum, H.J. (1972) A simple non-surgical technique to obtain unimplanted eggs from human uteri. *American Journal of Obstetrics and Gynecology*, **112**, 662–668.

Cruickshank, W. (1797) Experiments in which, on the third day after impregnation, the ova of rabbits were found in the Fallopian tube; and on the fourth day after impregnation in the uterus itself, with the first appearances of the foetus. *Philosophical Transactions*, pt 1, 197–214.

Cuneo, C.L. (1986) Studies of the physiology and biochemistry of early development in the preimplantation sheep embryo. PhD Thesis, Murdoch University, Western Australia.

Czlonkowska, M., Boyle, M.S. and Allen, W.R. (1985) Deep freezing of horse embryos. *Journal of Reproduction and Fertility*, **75**, 485–490.

Dalcq, A.M. (1957) *Introduction to General Embryology*, Oxford University Press.

Dalpra, L., Nocera, G., Tibiletti, M.G., Gramellini, F., Agosti, S. and Oldrini, A. (1986) Technical aspects and diagnostic problems of direct chromosome analysis using chorionic villus sampling in the first trimester. *Human Reproduction*, **1**, 103–106.

Dalton, A.J. (1972) RNA tumour viruses. Terminology and ultrastructural aspects of virion morphology and replication. *Journal of the National Cancer Institute*, **49**, 323–327.

Daniel, J.C. (ed.) (1971) *Methods in Mammalian Embryology*, Freeman, San Francisco.

Daniel, J.C. (ed.) (1978) *Methods in Mammalian Reproduction*, Freeman, San Francisco.

Daniel, J.C. and Chilton, B.S. (1978) Virus-like particles in embryos and female reproductive tract in *Development in Mammals*, Vol. 3 (ed. M.H. Johnson), North/Holland, Amsterdam, pp. 131–187.

Deansley, R. (1966) The endocrinology of pregnancy and foetal life in *Marshall's Physiology of Reproduction*, 3rd edn, Vol. 3, Chapter 32, (ed A.S. Parkes), Longman, Green and Co, London.

Depypere, H.T., McLaughlin, K.J., Seamark, R.F., Warnes, G.M. and Matthews, C.D. (1988) Comparison of zona cutting and zona drilling as techniques for assisted fertilization in the mouse. *Journal of Reproduction and Fertility*, **84** 205–211.

Dickmann, Z. (1971) Egg Transfer in *Methods in Mammalian Embryology* (ed. J.D. Daniel), Freeman, San Francisco, pp. 133–145.

Dokras, A. Sargent, I.L., Ross, C., Gardner, R.L. and Barlow, D.H. (1990) Trophectoderm biopsy in human blastocysts. *Human Reproduction*, **5**, 821–825.

Drost, M., Wright Jr., J.M., Cripe, W.S. and Richter, A.R. (1983) Embryo transfer in water buffalo (*Bubalus bubalis*). *Theriogenology*, **20**, 579–584.

Ducibella, T. (1977) Surface changes of the developing trophoblast cell in *Development in Mammals*, Vol. 1 (ed. M.H. Johnson), North/Holland, Amsterdam, New York, Oxford, pp. 5–30.

Ducibella, T. and Anderson, E. (1975) Cell shape and membrane changes in the eight-cell mouse embryo; pre-requisites for morphogenesis of the blastocyst. *Developmental Biology*, **47**, 45–58.

Ducibella, T., Albertini, D.F., Anderson, E. and Biggers, J.D. (1975) The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. *Developmental Biology*, **45**, 231–250.

Dvorak, M., Cech, S., Stasna, J., Tesarik, J. and Travnik, P. (1985) *The Differentiation of Preimplantation Mouse Embryos*, J.E. Purkyne University, Brno.

Edirisinghe, W.R. and Wales, R.G. (1984) Effect of parenteral administration of oestrogen and progesterone on the glycogen metabolism of mouse morulae-early blastocysts *in vivo*. *Journal of Reproduction and Fertility*, **72**, 67–73.

Edwards, R.G. (1965) Maturation *in vitro* of human ovarian oocytes. *Lancet*, **ii**, 926–929.

Edwards, R.G., Bavister, B.D. and Steptoe, P.C. (1969) Early stages of fertilization *in vitro* of human oocytes matured *in vitro*. *Nature*, **221**, 632–635.

Edwards, R.G., Steptoe, P.C. and Purdy, J. (1970) Fertilization and cleavage *in vitro* of preovulatory human oocytes. *Nature*, **227**, 1307–1309.

Edwards, R.G., Purdy, J., Steptoe, P.C. and Walter, D.E. (1981) The growth of human preimplantation embryos *in vitro*. *American Journal of Obstetrics and Gynecology*, **141**, 408–416.

Ellington, J.E., Farrell, P.B., Simkin, M.E., Foote, R.H., Goldman, E.E. and McGrath, A.B. (1990) Development and survival after transfer of cow embryos cultured from 1–2 cell to morulae or blastocyst in rabbit oviducts or in a simple me-

dium with bovine oviduct epithelial cells. *Journal of Reproduction and Fertility*, **89**, 293–299.

Emery, A.E.H. (1984) *An Introduction to Recombinant DNA*, John Wiley, Chichester, UK.

Epstein, C.J. (1975) Gene expression and macromolecular synthesis during preimplantation development. *Biology of Reproduction*, **12**, 82–105.

Evans, M.J. and Kaufman, M.H. (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature*, **292**, 154–156.

Eyestone, W.H. and First, N.L. (1989) Co-culture of early cattle embryos to the blastocyst stage with oriductal tissue or in conditioned medium. *Journal of Reproduction and Fertility*, **85**, 715–720.

Eyestone, W.H. and First, N.L. (1989) Culture of early bovine embyros with oviductal tissue or in conditioned medium. *Journal of Reproduction and Fertility*, **85**, 415–420.

Eyestone, W.H., Jones, J.M. and First, N.L. (1991) Some factors affecting the efficacy of oviduct-tissue conditioned medium for the culture of early bovine embryos. *Journal of Reproduction and Fertility*, **92**, 59–64.

Fabro, S., McLachlan, J.A. and Dames, N.M. (1984) Chemical exposure of embryos during the preimplantation stages of pregnancy: mortality rate and intrauterine development. *American Journal of Obstetrics and Gynecology*, **148**, 929–938.

Fawcett, D.W., Wislocki, G.B. and Waldo, C.M. (1947) The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *American Journal of Anatomy*, **81**, 413–444.

Fehilly, C.B. and Willadsen, S.M. (1986) Embryo manipulation in farm animals in *Oxford Reviews of Reproductive Biology*, Vol. 8 (ed. J. Clark), Oxford University Press, pp. 379–413.

Fehilly, C.B., Willadsen, S.M. and Tucker, E.M. (1984) Experimental chimaerism in sheep. *Journal of Reproduction and Fertility*, **70**, 347–351.

First, N.L. (1990) New Animal breeding techniques and their application. *Journal of Reproduction and Fertility*, Suppl. 41, 525–530.

Fishel, S., Antinori, S., Jackson, P., Johnson, J., Lisi, F., Chiariello, F. and Versaci, C. (1990) Twin birth after sub-zonal insemination. *Lancet*, **335**, 722–723.

Flach, G. Johnson, M.H., Braude, P.R., Taylor. R.A.S. and Bolton, V.N. (1982) The transition from maternal to embryonic control in the 2-cell mouse embryo. *Embo Journal*, **1**, 681–686.

Flint, A.P.F., Renfree, M.B. and Weir, B.J. (eds) (1981) *Embryonic Diapause in Mammals*, Journal of Reproduction and Fertility, Suppl. 29.

Frei, R.E., Schultz, G.A. and Church, R.B. (1989) Qualitative and quantitative changes in protein synthesis occur at the 8-16-cell stage of embryogenesis in the cow. *Journal of Reproduction and Fertility*, **86**, 637–641.

Fridhandler, L. (1961) Pathways of glucose metabolism in fertilized rabbit ova at various preimplantation stages. *Experimental Cell Research*, **22**, 303–316.

Fridhandler, L., Hafez, E.S. and Pincus, G. (1957) Developmental changes in the respiratory activity of rabbit ova. *Experimental Cell Research*, **13**, 132–139.

Fugger, E.F., Bustillo, M., Dorfmann, A.D. and Schulman, J.D. (1991) Human preimplantation embryo cryopreservation: selected aspects. *Human Reproduction*, **6**, 131–135.

Fujii, J.T. and Martin, G.R. (1980) Incorporation of teratocarcinoma stem cells into the blastocyst by aggregation with cleavage stage mouse embryos. *Developmental Biology*, **74**, 239–244.

Fukui, Y., McGowan, L.T., James, R.W., Pugh, P.A. and Tervit, H.R. (1991) Factors affecting the *in vitro* development to blastocysts of bovine oocytes matured and fertilized *in vitro*. *Journal of Reproduction and Fertility*, **92**, 125–131.

Funaki, K. and Mikamo, K. (1983) Developmental-stage-dependent teratogenic effects of maternal spontaneous diabetes in the Chinese hamster. *Journal of the American Diabetes Association*, **32**, 637–643.

Gamow, E. and Daniel, J.C. (1970) Fluid transport in the rabbit blastocyst. *Wilhelm Roux Archiv*, **164**, 261–278.

Gandolfi, F., Brevini, T.A.L. and Moor, R.M. (1989) Effect of oviduct environment on embronic development. *Journal of Reproduction and Fertility*, Suppl. 38, 107–115.

Gardner, R.L. (1968) Mouse chimaeras obtained by the injection of cells into the blastocyst. *Nature*, **220**, 596–597.

Gardner, R.L. (1971) Manipulations on the blastocyst in *Advances in the Biosciences*, Vol. 6 (ed. G. Raspe), Schering Symposium on Intrinsic and Extrinsic Factors in Early Mammalian Development, Venice 1970, Pergamon Press, Vieweg, pp. 279–296.

Gardner, R.L. (1975) Analysis of determination and differentiation in the early mammalian embryo using inter- and intra-specific chimaeras in *Developmental Biology of Reproduction, Symposium*

of the Society for Developmental Biology, Vol. 33, pp. 207–236, Academic Press, New York.

Gardner, R.L. (1978) The relationship between cell lineage and differentiation in the early mouse embryo in *Genetic Mosaics and Cell Differentiation: Results and Problems in Cell Differentiation*, Vol. 9 (ed. W. Gehring), Springer-Verlag, Berlin.

Gardner, R.L. (1982) Manipulation of Development in *Reproduction in Mammals*, 2nd edn, book 2 (eds C.R. Austin and R.V. Short), Cambridge University Press, pp. 159–180.

Gardner, R.L. and Edwards, R.G. (1968) Control of the sex ratio at full term in the rabbit by transferring sexed blastocysts. *Nature*, **218**, 346–348.

Gardner, R.L. and Johnson, M.H. (1973) Investigation of early mammalian development using interspecific chimaeras between rat and mouse. *Nature, New Biology*, **246**, 86–89.

Gardner, R.L. and Munro, A.J. (1974) Successful construction of chimaeric rabbit. *Nature*, **250**, 146–147.

Gartler, S.M. and Cole, R.E. (1981) Recent developments in the study of mammalian X-chromosome inactivation in *Mechanisms of Sex Differentiation in Animals and Man*, (eds C.R. Austin and R.G. Edwards), Academic Press, London, pp. 113–143.

Gegenbaur, C. (1861) Ueber den Bau und die Entwickelung der Wirbelthiereier mit partieller Dottertheilung. *Archives of Anatomy and Physiology*, Lpz, 491.

Glover, D.M. (1984) *The Mechanics of DNA Manipulation*, Chapman and Hall, London.

Godke, R.A., Beetem, D.D. and Burleigh, D.W. (1990) A method for zona pellucida drilling using a compact nitrogen laser. *VII World Congress on Human Reproduction, Helsinki*, Abstract 258.

Gordon, J.W. and Talansky, B.E. (1986) Assisted fertilization by zona drilling: a mouse model for correction of oligospermia. *Journal of Experimental Zoology*, **239**, 347–354.

Gordon, J.W., Scangos, C.A., Plotkin, D.J., Barbosa, J.A. and Ruddle, F.H. (1980) Genetic transformation of mouse embryos by microinjection of purified DNA. *Proceedings of the National Academy of Sciences of the USA*, **77**, 7380–7384.

Gordon, J.W., Grunfeld, L., Garrisi, G.J., Talansky, B.E., Richards, C. and Laufer, N. (1988) Fertilisation of human oocytes by sperm from infertile males after zone pellucida drilling. *Fertility and Sterility*, **50**, 68–73.

Gosden, S.R. and Gosden, C.M. (1985) Recombinant DNA technology in prenatal diagnosis in *Oxford Reviews of Reproductive Biology*, (ed. J.R. Clarke), Oxford University Press, **7**, 73–117.

Gott, A.L., Hardy, K., Winston, R.M.L. and Leese, H.J. (1990) Non-invasive measurement of pyruvate and glucose uptake and lactate production by single human preimplantation embryos. *Human Reproduction*, **5**, 104–108.

de Graaf, R. (1672) *De mulierium organis generations inservientibus*. Tractus novus. Lugdoni, Batay.

Graham, C.F. (1970) Parthenogenetic mouse blastocytes. *Nature*, **226**, 165–167.

Graham, C.F. (1974) The production of parthenogenetic mammalian embryos and their use in biological research. *Biological Reviews*, **49**, 399–422.

Graham, F.L. and Van Der Eb, A.J. (1973) Transformation of rat cells by DNA of human Adenovirus 5. *Virology*, **54**, 536–539.

Grant, P. (1978) *Biology of Developing Systems*, Holt, Rinehart and Winston, New York.

Gray, A.P. (1954) *Mammalian Hybrids*, Commonwealth Agricultural Bureaux, Farnham Royal, England.

Greve, T., Callesen, H. and Hyttel, P. (1989) Follicular correlates with *in vitro* fertilization in cattle. *Journal of Reproduction and Fertility*, Suppl. 38, 117–126.

Griffin, D.K., Wilton, L.J., Handyside, A.H., Winston, R.M.L. and Delaney, J.D.A. (1992) Dual fluorescent in situ hybridisation for simultaneous detection of X-chromosome-specific and Y-chromosome specific probes for the sexing of human preimplantation embryonic nuclei. *Human Genetics*, **89**, 18–22.

Griffith, F. (1928) Significance of pneumococcal types. *Journal of Hygiene*, **27**, 113–159.

Griffiths, M., Mackintosh, D.L. and Coles, R.E.A. (1969) The mammary gland of the echidna, *Tachyglossus aculeatus*, with observations on the incubation of the egg and on the newly hatched young. *Journal of Zoology, London*, **15**, 376–386.

Gropp, A., Tattenborn, U. and von Lehman, E. (1970) Chromosomenvariation vom Robertsons'-schen Typus bei der Tabakmaus *M. poschiavinus* und ihren Hybriden mit der Laboratoriumsmaus. *Cytogenetics and Cell Genetics*, **9**, 9–23.

Gropp, A., Putz, B. and Zimmerman, U. (1976) Autosmal monosomy and trisomy causing

developmental failure in *Developmental Biology and Pathology*, (eds A. Gropp and K. Benirschke), Springer-Verlag, Berlin, pp. 177–192.

Guillomot, M., Flechon, J-E. and Wintenberger-Torres, S. (1982) Cytochemical studies of uterine and trophoblast surface coats during blastocyst attachment in the ewe. *Journal of Reproduction and Fertility*, **65**, 1–8.

Gulyas, B.J., Wood, M. and Whittingham, D.G. (1984) Fusion of oocytes and development of fusion products in the mouse. *Developmental Biology*, **101**, 246–250.

Gurdon, J.B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *Journal of Embryology and Experimental Morphology*, **10**, 622–640.

Gurdon, J.B. (1968) Transplanted nuclei and cell differentiation. *Scientific American*, **219**, Dec., 24–35.

Gurdon, J.B. and Uehlinger, V. (1966) 'Fertile' intestinal nuclei. *Nuclei*, **210**, 1240–1241.

Gwatkin, R.B.L. (1966) Defined media and the development of mammalian eggs *in vitro*. *Annals of the New York Academy of Sciences*, **139**, 79–90.

Hafez, E.S.E. and Blandau, R.J. (eds) (1969) *The Mammalian Oviduct; Comparative Biology and Methodology*, University of Chicago Press, Chicago.

Hamilton, W.J. and Laing, J.A. (1946) Development of the egg of the cow up to the stage of blastocyst formation. *Journal of Anatomy*, **80**, 194–204.

Hammer, R.E., Brinster, R.L., Rosenfeld, M.G., Evans, R.M. and Mayo, K.E. (1985) Expression of human growth hormone-releasing factor in transgenic mice results in increased somatic growth. *Nature*, **315**, 413–416.

Hammond Jr., J. (1949) Recovery and culture of tubal mouse ova. *Nature*, **163**, 28.

Handyside, A.H., Kontogianni, H., Hardy, K. and Winston, R.M.L. (1990) Pregnancies from biopsied human pre-implantation embryos sexed by Y-specific DNA amplification. *Nature*, **344**, 768–770.

Hansel, W. and Weir, B.J. (eds) (1990) *Genetic Engineering of Animals. Journal of Reproduction and Fertility*, Suppl. 41, Journal of Reproduction and Fertility Ltd, Cambridge.

Hardy, K. and Handyside, A.H. (1992) Biopsy of cleavage stage human embryos and diagnosis of single gene defects by DNA amplification. *Archives of Pathology Laboratory Medicine*, **116**, 388–3982.

Hardy, K., Martin, K.L., Leese, H.J., Winston, R.M.L. and Handyside, A.H. (1990) Human preimplantation development *in vitro* is not adversely affected by biopsy at the 8-cell stage. *Human Reproduction*, **5**, 708–714.

Hartman, C.G. (1916) Studies on the development of the opossum (*Didelphis virginiana* L.). I. History of first cleavage. II. Formation of the blastocyst. *Journal of Morphology*, **27**, 1–83.

Hartshorne, G.M., Elder, K., Crow, J., Dyson, H. and Edwards, R.G. (1991) The influence of *in vitro* development upon post-thaw survival and implantation of cryopreserved human blastocysts. *Human Reproduction*, **6**, 136–141.

Heape, W. (1891) Preliminary note on the transplantation and growth of mammalian ova within a uterine foster-mother. *Proceedings of the Royal Society of London Series B*, **48**, 457–458.

Heape, W. (1897) Further note on the transplantation and growth of mammalian ova within uterine foster-mother. *Proceedings of the Royal Society of London Series B*, **62**, 178–183.

Hearn, J.P. (1984) Scientific report of the Zoological Society of London, 1982–1983, pp. 30–31. *Journal of Zoology, London*, **204**, 1–99.

Hearn, J.P. and Summers, P.M. (1986) Experimental manipulation of embryonic implantation in the marmoset monkey and exotic equids. *Theriogenology*, **25**, 333–341.

Heuser, C.H. and Streeter, G.L. (1929) Early stages in the development of pig embryos from the period of initial cleavage to the time of appearance of limb-buds. *Carnegie Institution of Washington Contributions to Embryology*, No. **109**, 1–30.

Heyman, Y., Ménézo, Y., Chesné, P., Camous, S. and Garnier, V. (1987) *In vitro* cleavage of bovine and ovine early embryos: improved development using coculture with trophoblastic vesicles. *Theriogenology*, **27**, 59–68.

Hill, J.P. (1910) The early development of the marsupialia, with special reference to the native cat (*Dasyurus viverrinus*). *Quarterly Journal of Microscopical Science*, **56**, 1–134.

Hillman, N., Sherman, M.I. and Graham, C. (1972) The effect of spatial arrangements on cell determination during mouse development. *Journal of Embryology and Experimental Morphology*, **28**, 263–278.

Hiromoto, Y. (1962) Microinjection of the live sper-

matozoa into sea urchin eggs. *Experimental Cell Research*, **27**, 416–426.

Hoppe, P.C. and Illmensee, K. (1977) Microsurgically produced homozygous-diploid uniparental mice. *Proceedings of the National Academy of Sciences of the USA*, **74**, 5657–5661.

Hoppe, P.C. and Illmensee, K. (1982) Full-term development after transplantation of parthenogenetic embryonic nuclei into fertilized mouse eggs. *Proceedings of the National Academy of Sciences of the USA*, **79**, 1912–1916.

Hsu, Y.-C. (1971) Post blastocyst differentiation *in vitro*. *Nature*, **231**, 100–102.

Hughes, R.L. (1977) Egg membranes and ovarian function during pregnancy in monotremes and marsupials, in *Reproduction and Evolution*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), Australian Academy of Science, Canberra, pp. 281–291.

Hughes, R.L. (1982) Reproduction in the Tasmanian devil (*Sarcophilus harrisii*) (Dasyuridae, Marsupialia), in *Carnivorous Marsupials*, (ed. M. Archer), Surrey Beatty, Sydney, pp. 49–63.

Hughes, R.L. (1984) Structural adaptations of the eggs and the fetal membranes of monotremes and marsupials for respiration and metabolic exchange in *Respiration and Metabolism of Embryonic Vertebrates*, (ed. R.S. Seymour), Junk Publishers, Dordrecht.

Hughes, R.L. and Carrick, F.N. (1978) Reproduction in female monotremes in *Monotreme Biology*, Vol 20 (ed. M.L. Augee), Royal Zoological Society, NSW, Australia, pp. 233–253.

Hughes, R.L. and Hall, L.S. (1984) Embryonic development in the common bushtail possum *Trichosurus vulpecula*, in *Possums and Gliders*, (eds A.P. Smith and I.D. Hume), Australian Mammal Society, Sydney, pp. 197–212.

Hunter, R.H.F. (1980) *Physiology and Technology of Reproduction in Female Domestic Animals*, Academic Press, London.

Illmensee, K. and Hoppe, P.C. (1981) Nuclear transplantation in Mus musculus: developmental potential of nuclei from preimplantation embryos. *Cell*, **23**, 9–18.

Jackson, I.J. (1989) The mouse in *Genes and Embryos*, (eds D.M. Glover and B.D. Hames), IRL Press, Oxford.

Jaenisch, R. (1984) Retroviruses and mammalian development in *Genetic Manipulation: Impact on Man and Society*, (eds W. Arber, K. Illmensee, W.J. Peacock and P. Starlinger), Cambridge University Press, pp. 199–208.

Jaenisch, R. and Mintz, B. (1974) Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. *Proceedings of the National Academy of Sciences of the USA*, **71**, 1250–1254.

Jaenisch, R., Harbers, K., Schnieke, A., Lohler, J., Chumakov, I., Jähner, D., Grotkopp, D. and Hoffman,, E. (1983) Germline integration of Malony Murine Leukemia virus at the mov 13 locus leads to recessive lethal mutation and early embryonic death. *Cell*, **32**, 209–216.

Jähner, D. and Jaenisch, R. (1980) Integration of Molony leucaemia virus into the germ line of mice: correlation between site of integration and virus activation. *Nature*, **287**, 456–458.

Jansen, R.P.S. and Anderson, J.C. (1987) Catheterization of the fallopian tubes from the vagina. *Lancet*, **2**, 309–310.

Jansen, R.P.S., Anderson, J.C. and Sutherland, P.D. (1988) Nonoperative embryo transfer to the fallopian tube. *New England Journal of Medicine*, **319**, 288–291.

Johnson, M.H. (1981) The molecular and cellular basis of preimplantation mouse development. *Biological Reviews*, **56**, 456–458.

Johnson, M.H. (1985) Manipulation of early mammalian development: what does it tell us about cell lineages?, in *Developmental Biology: A Comprehensive Synthesis*, (ed. L. Browder), Plenum Press, New York, pp. 27–48.

Johnson, M.H., Pratt, H.P.M. and Handyside, A.H. (1981) The generation and recognition of positional information in the preimplantation mouse embryo in *Cellular and Molecular Asplects of Implantation*, (eds S.R. Glasser and D.W. Bullock), Plenum Press, New York, pp. 55–74.

Johnson, M.H., McConnell, J. and Van Blerkom, J. (1984) Programmed development in the mouse embryo. *Journal of Embryology and Experimental Morphology*, **83**, (Suppl.) 197–231.

Jones, K.W., Singh, L. and Edwards, R.G. (1987) The use of probes for the Y chromosome in preimplantation embryo cells. *Human Reproduction*, **5**, 439–445.

Kasai, M., Komi, J.H., Takahomo, A., Tsudera, H., Sakuri, T. and Machida, T. (1990) A simple method for mouse embryo cyropreservation in a low toxicity vitrification solution, without appreciable loss of viability. *Journal of Reproduction and Fertility*, **89**, 91–97.

Kaufman, M.H. (1983) *Early Mammalian Development: Parthenogenetic Studies*, Cambridge University Press.

Kaufman, M.H., Guc-Cubrilo, M. and Lyon, M.F. (1978) X-chromosome inactivation in diploid parthenogenetic mouse embryos. *Nature*, **271**, 547–549.

Kelly, S.J. (1975) Studies on the potency of early cleavage blastomeres of the mouse in *The Early Development of Mammals* (eds M. Balls and A.E. Wild), Cambridge University Press, pp. 97–106.

Kelly, S.J. (1979) Studies on the developmental potential of 4- and 8-cell stage mouse embryos. *Journal of Zoology*, **200**, 365–376.

Khurana, N.K. (1987) *Interactions between the embryo and its environment during the early stages of pregnancy*, PhD Thesis, Murdoch University, Western Australia.

King, W.A., Niar, A., Betteridge, K.J. and Guay, P. (1988) Nucleolus organizer regions and nucleoli in preattachment bovine embryos. *Journal of Reproduction and Fertility*, **82**, 87–95.

Kirby, D.R.S. (1960) Development of mouse eggs beneath the kidney capsule. *Nature*, **187**, 707–708.

Kirby, D.R.S. (1963a) Development of the mouse blastocyst transplanted to the spleen. *Journal of Reproduction and Fertility*, **5**, 1–12.

Kirby, D.R.S. (1963b) The development of mouse blastocysts transplanted to the scrotal and cryptorchid testis. *Journal of Anatomy*, **97**, 119–130.

Kirby, D.R.S. (1967) Ectopic autografts of blastocysts in mice maintained in delayed implantation. *Journal of Reproduction and Fertility*, **14**, 515–517.

Kola, I., Lacham, O., Jansen, R.P.S., Turner, M. and Trounson, A. (1990) Chromosomal analysis of human oocytes fertilized by microinjection of spermatozoa into the perivitelline space. *Human Reproduction*, **5**, 575–577.

Krishnan, R.S. and Daniel, J.C. (1967) 'Blastokinin' inducer and regulator of blastocyst development in the rabbit uterus. *Science*, **158**, 490–492.

Krishnan, R.S. and Daniel, J.C. (1968) Composition of 'blastokinin' from rabbit uterus. *Biochimica et Biophysica Acta*, **168**, 579–582.

Kuehl, T.J., Clayton, O. and Reyes, P.S. (1984) Live birth following *in vitro* fertilization and embryo transfer in a non-human primate. *American Journal of Primatology*.

Kuzan, F. and Wright, R. (1982) Observations on the development of bovine morulae on various cellular and non-cellular substrata. *Journal of Animal Science*, **54**, 811–816.

Lambeth, V.A., Looney, C.R., Voekel, S.A., Jackson, D.A., Hill, K.G. and Godke, R.A. (1983) Microsurgery on bovine embryos at the morula stage to produce monozygotic twin calves. *Theriogenology*, **20**, 85–95.

Land, R.B. and Wilmut, I. (1987) Gene transfer and animal breeding. *Theriogenology*, **27**, 169–179.

Larsson, E., Nilsson, B.O., Sundstrom, P. and Widehn, S. (1981) Morphological and microbiological signs of endogenous C-virus in human oocytes. *International Journal of Cancer*, **28**, 551–557.

Lassalle, B. and Testart, J. (1988) Human sperm injection into the perivitilline space (SI-PVS) of hamster oocytes: effect of sperm pretreatment by calcium ionophore A23187 and freeze-thawing on the penetration rate and polyspermy. *Gamete Research*, **20**, 310–311.

Lawitts, J.A. and Biggers, J.D. (1991) Optimization of mouse embryo culture media using simplex methods. *Journal of Reproduction and Fertility*, **91**, 543–556.

Laws-King, A., Trounson, A., Sathananthan, H. and Kola, I. (1987) Fertilization of human oocytes by microinjection of a single spermatozoan under the zona pellucida. *Fertility and Sterility*, **48**, 637–642.

Lazendorf, S.E., Malone, M.K., Veek, L.L., Slusser, J., Hodgen, G.D. and Rosenwaks, Z. (1988) A preclinical evaluation of pronuclei formation by microinjection of human spermatozoa into human oocytes. *Fertility and Sterility*, **49**, 835–842.

Lederberg, J. and Tatum, E.L. (1946) Gene recombination in *Escherichia coli*. *Nature*, **158**, 558.

Leese, H.J., Hooper, M.A.K., Edwards, R.G. and Ashwood-Smith, M.J. (1986) Uptake of pyruvate by early mammalian embryos determined by a non-invasive technique. *Human Reproduction*, **1**, 181–182.

Leese, H.J., Humpherson, P.G., Hardy, K., Hopper, M.A.K., Winston, R.M.L. and Handyside, A.H. (1991) Profiles of hypoxanthine guanine phosphoribosyl transferase and adenine phosphoribosyl transferase activities measured in single preimplantation human embryos by high performance liquid chromatography. *Journal of Reproduction and Fertility*, **91**, 197–202.

Lehn-Jensen, H. and Willadsen, S.M. (1983) Deep

freezing of cow 'half' and 'quarter' embryos. *Theriogenology*, **19**, 49–54.

Leonard, M., Kirszenbaum, M., Cotinot, C., Chesne, P., Heyman, Y., Stinnakre, M.G., Bishop, C., Delouis, C., Vaiman, M. and Fellous, M. (1987) Sexing bovine embryos using Y chromosome specific DNA problem. *Theriogenology*, **27**, 248.

Lewis, W.H. and Gregory, P.W. (1929) Cinematographs of living developing rabbit eggs. *Science*, **69**, 226–229.

Loskutoff, N.M., Raphael, B.L., Nemec, L.A., Wolfe, B.A., Howard, J.G. and Kraemer, D.C. (1990) Reproductive anatomy, manipulation of ovarian activity and non-surgical embryo transfer in suni (*Neotragus moschatus zuluensis*). *Journal of Reproduction and Fertility*, **88**, 521–532.

Luckett, W.P. (1977) Ontogeny of amniote fetal membranes and their application to phylogeny in *Major Patterns in Vertebrate Evolution*, (eds M.K. Hecht, P.C. Goody and B.M. Hecht), Plenum Press, New York, pp. 439–516.

Lutwak-Mann, C. (1954) Some properties of the rabbit blastocyst. *Journal of Embryology and Experimental Morphology*, **2**, 1–13.

Lutwak-Mann, C. (1959) Biochemical approach to the study of ovum plantation in the rabbit in *Implantation of Ova. Memoirs of the Society for Endocrinology*, No. 6 (ed. P. Eckstein), Cambridge University Press, p. 97.

Lutwak-Mann, C. (1960) Some properties of the early embryonic fluids in the rabbit. *Journal of Reproduction and Fertility*, **1**, 316–317.

Lyon, M.F. (1961) Gene action in the X-chromosomes of the mouse (*Mus musculus* L). *Nature*, **190**, 372–373.

Lyon, M.F. (1972) X-chromosome inactivation and developmental patterns in mammals. *Biological Reviews*, **47**, 1–35.

Lyon, M.F., Glenister, P.H. and Lamoreux, M.L. (1975) Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. *Nature*, **258**, 620–622.

McCaffrey, C., McEvoy, T.G., Diskin, M.G., Gwazdaucas, F.C., Kane, M.T. and Sreenan, J.M. (1991) Successful co-culture of 1- to 4-cell cattle ova to the morula or blastocyst stage. *Journal of Reproduction and Fertility*, **91**, 119–124.

McGrath, J. and Solter, D. (1983) Nuclear transplantation in the mouse embryo using microsurgery and cell fusion. *Science*, **220**, 1300–1302.

McGrath, J. and Solter, D. (1984a) Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development *in vitro*. *Science*, **226**, 1317–1319.

McGrath, J. and Solter, D. (1984b) Maternal T^{hp} lethality in the mouse is a nuclear, not cytoplasmic, defect. *Nature*, **308**, 550–551.

McKiernan, S.H., Bavister, B.D. and Tasca, R.J. (1991) Energy substrate requirements for *in vitro* development of hamster 1- and 2-cell embryos to the blastocyst stage. *Human Reproduction*, **6**, 64–75.

McLaren, A. (1973) Blastocyst activation in *The Regulation of Mammalian Reproduction*, (eds S. Segal, R. Crozier, P. Corfman and P. Cundliffe), C.C. Thomas, Springfield, pp. 321–328.

McLaren, A. (1976a) Genetics of the early mouse embryo. *Annual Reviews of Genetics*, **10**, 361–388.

McLaren, A. (1976b) *Mammalian Chimaeras*, Cambridge University Press.

McLaren, A. (1981) Mammalian chimaeras in *Well-being of Mankind and Genetics. Proceedings of the 14th International Congress of Genetics*, Vol. 1, book 2 (ed. D.K. Belyaev), MIR Publishers, Moscow, pp. 142–150.

McLaren, A. (1982) The embryo in *Reproduction in Mammals*, Book 2, *Embryonic and Fetal Development* (eds C.R. Austin and R.V. Short), Cambridge University Press, pp. 1–25.

McLaren, A. (1985) Prenatal diagnosis before implantation: opportunities and problems. *Prenatal Diagnosis*, **5**, 85–90.

McLaren, A. and Biggers, J.D. (1958) Successful development and birth of mice cultivated as early embryos. *Nature*, **182**, 877–878.

Magnuson, T. and Epstein, C.J. (1981) Genetic control of very early mammalian development. *Biological Reviews*, **56**, 369–408.

Magnusson, C., Hillensjö, T., Hamberger, L. and Nilsson,, L. (1986) Oxygen consumption by human oocytes and blastocytes grown *in vitro*. *Human Reproduction*, **1**, 183–184.

Malter, H. and Cohen, C.J. (1989) Partial zona dissection of human oocytes: A non-traumatic method using micromanipulation to assist zona pellucida penetration. *Fertility and Sterility*, **51**, 139–148.

Mann, J.R. (1988) Full-term development of mouse eggs fertilized by a spermatozoan micro-injected under the zona pellucida. *Biology of Reproduction*, **38**, 1077–1083.

Mann, J.R. and Lovell-Badge, R.H. (1984) Inviability of parthenogenones is determined by

pronuclei, not egg cytoplasm. *Nature*, **310**, 66–67.

Markert, C.L. (1982) Parthenogenesis, homozygosity and cloning in mammals. *Journal of Heredity*, **73**, 390–397.

Markert, C.L. (1983) Fertilisation of mammalian eggs by sperm injection. *Journal of Experimental Zoology*, **228**, 195–201.

Massey, J.M. (1990) Animal production industry in the year 2000 AD. *Journal of Reproduction and Fertility*, Suppl. 41, pp. 199–208.

Maxwell, D., Lilford, R., Morsman, J., Rodeck, C., Old, J. and Thein, S. (1985) Direct DNA analysis for diagnosing fetal sickle status in first trimester chorion tissue. *Journal of Obstetrics and Gynaecology*, **5**, 133–135.

Mayer, J.F. and Fritz, H.I. (1974) The culture of preimplantation rat embryos and the production of allophenic rats. *Journal of Reproduction and Fertility*, **39**, 1–9.

Mintz, B. (1962) Formation of genotypically mosaic mouse embryos (abstract). *American Zoologist*, **2**, 432.

Mintz, B. (1964) Formation of genotypically mosaic mouse embryos and early development of 'lethal (t^{12}/t^{12})-normal' mosaics. *Journal of Experimental Zoology*, **157**, 273–292.

Mintz, B. (1974) Gene control of mammalian differentiation. *Annual Review of Genetics*, **8**, 411–470.

Mintz, B. and Baker, W.W. (1967) Normal mammalian muscle differentiation and gene control of isocitrate dehydrogenase. *Proceedings of the National Academy of Sciences of the USA*, **58**, 592–598.

Mintz, B. and Illmensee, K. (1975) Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proceedings of the National Academy of Sciences of the USA*, **72**, 3585–3589.

Mittwoch, U. (1978) Parthenogenesis. *Journal of Medical Genetics*, **15**, 165–181.

Modlinski, J.A. (1978) Transfer of embryonic nuclei to fertilized mouse eggs and development of tetraploid blastocysts. *Nature*, **273**, 466-467.

Modlinski, J.A. (1981) The fate of inner cell mass and trophectoderm nuclei transplanted to fertilized mouse eggs. *Nature*, **292**, 342-343.

Mohr, L.R., Trounson, A. and Freeman, L. (1985) Deep-freezing and transfer of human embryos. *Journal of In Vitro Fertilization and Embryo Transfer*, **2**, 1–10.

Monk, M. and Harper, M. (1979) Sequential X-chromosomes inactivation coupled with cellular differentiation in early mouse embryos. *Nature*, **281**, 311–313.

Moore, M.W., Adams, C.E. and Rowson, L.E.A. (1968) Developmental potential of single blastomeres of the rabbit egg. *Journal of Reproduction and Fertility*, **17**, 527–531.

Moore, M.W., Polge, C. and Rowson, L.E.A. (1969) The survival of single blastomeres of pig eggs transferred to recipient gilts. *Australian Journal of Biological Sciences*, **22**, 979–982.

Mortimer, D., Curtis, E.F. and Dravland, J.E. (1886) The use of strontium-substituted media for capacitating human spermatozoa: an improved method for the zona-free hamster egg penetration test. *Fertility and Sterility*, **46**, 97–103.

Moustafa, L.A. (1974) Chimaeric rabbits from embryonic cell transplantation. *Proceedings of the Society for Experimental Biology and Medicine*, **147**, 485–488.

Muggleton-Harris, A.L., Whittingham, D.G. and Wilson, L. (1982) Cytoplasmic control of preimplantation development *in vitro* in the mouse. *Nature*, **299**, 460–462.

Muggleton-Harris, A.L., Findlay, I. and Whittingham, D.G. (1990) Improvement of the culture conditions for the development of human preimplantation embryos. *Human Reproduction*, **5**, 217–220.

Mukherjee, A.B. and Cohen, M.M. (1970) Development of normal mice by *in vitro* fertilization. *Nature*, **228**, 472–473.

Mulnard, J.G. (1965) Studies of regulation of mouse ova *in vitro* in *Preimplantation Stages of Pregnancy*, (eds G.E.W. Wolstenholme and M. O'Connor), Churchill, London, pp. 123–138.

Mulnard, J.G. (1973) Formation de blastocystes chimerique par fusion d'embryons de rat et de souris au stage VIII. *Comptes Rendues de l'Academie des Sciences de Paris*, **276**, 379–381.

Mulnard, J.G. (1986) An historical survey of some basic contributions to casual mammalian embryology. *Human Reproduction*, **1**, 373–380.

Murray, F.A., Bazer, F.W., Wallace, H.D. and Warnick, A.C. (1972) Quantitative and qualitative variation to the secretion of protein by the porcine uterus during the estrus cycle. *Biology of Reproduction*, **7**, 314–320.

Mystkowska, E.T. (1975) Development of mouse-bank vole chimaeric embryos. *Journal of Embryology and Experimental Morphology*, **23**, 395–405.

Naaktgeborn, N., Devrosy, P., Wisanto, A., Traey, E. and Van Stiertgehem (1986) Endocrine

profiles in early pregnancies with delayed implantation. *Human Reproduction*, **1**, 9–14.

Nasr-Esfahani, M.H., Aitken, J.R. and Johnson, M.H. (1990) Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed *in vitro* or *in vivo*. *Development*, **109**, 501–507.

Newmark, P. (1983) Cloning technique under cloud. *Nature*, **303**, 363.

Newmark, P. (1984) Ilmensee inquiry. *Nature*, **307**, 673.

Ng, S.C., Bongso, T.A., Ratnam, S.S., Sathananthan, H., Chan, C.L.K., Wong, P.C., Hagglund, L., Anandakumar, C., Wong, Y.C. and Goh, V.H.H. (1988) Pregnancy after transfer of multiple sperm under the zona. *Lancet*, **ii**, 790.

Nicholas, J.S. (1942) Experiments on developing rats. IV. The growth and differentiation of eggs and egg cylinders when transplanted under the kidney capsule. *Journal of Experimental Zoology*, **90**, 41–64.

Nicholas, J.S. (1947). Experimental approaches to problems of early development in the rat. *Quarterly Reviews of Biology*, **22**, 179–195.

Nicholas, J.S. and Hall, B.V. (1942) Experiments on developing rats. II. The development of isolated blastocysts and fused eggs. *Journal of Experimental Zoology*, **90**, 441–458.

Nichols, J. and Gardner, R.L. (1984) Heterogeneous differentiation of external cells in individual isolated early mouse inner cell masses in culture. *Journal of Embryology and Experimental Morphology*, **80**, 225–240.

Nordenskiöld, E. (1928) *The History of Biology*, Tudor, New York.

Norris, M.L., Barton, S.C. and Surani, M.A.H. (1985) A quantitative comparison of protein synthesis in the preimplantation embryos of four rodent species (mouse, rat, hamster and gerbil). *Gamete Research*, **12**, 313–316.

Nossal, G.J.V. (1985) *Reshaping Life*, Cambridge University Press.

Notarianni, E., Laurie, S., Moor, R.M. and Evans, M.J. (1990) Maintenance and differentiation in culture of pluripotential cell lines from pig blastocysts. *Journal of Reproduction and Fertility*, Suppl. 41, 51–56.

Odawara, Y. and Lopata, A. (1989) A zona opening procedure for improving *in vitro* fertilization at low sperm concentrations: a mouse model. *Fertility and Sterility*, **51**, 699–704.

Oguri, N. and Tsutsumi, Y. (1982) Non-surgical transfer of equine embryos in *In Vitro Fertilization and Embryo Transfer*, (eds E.S.E. Hafez and K. Semm), MTP Press, Lancaster, pp. 287–295.

Ozil, J.-P., Heyman, Y. and Renard, J.-P (1982) Production of monozygotic twins by micromanipulation and cervical transfer in the cow. *Veterinary Record*, **10**, 126–127.

Palmiter, R.D. and Brinster, R.L. (1984) Making a bigger mouse in *Genetic Manipulation: Impact on Man and Society*, (eds W. Arber, K. Illmensee, W.J. Peacock and P. Starlinger), Cambridge University Press, pp. 187–197.

Palmiter, R.D., Brinster, R.L., Hammer, R.E., Trumbauer, M.E., Rosenfeld, M.G., Birnberg, N.C. and Evans, R.M. (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature*, **300**, 611–615.

Palmiter, R.D., Norstedt, G., Gelinas, R.E., Hammer, R.E. and Brinster, R.L. (1983) Metallothionein-human GH fusion genes stimulate growth in mice. *Science*, **222**, 809–814.

Papaioannou, V.E. (1986) Diapause in mouse blastocysts transferred to oviducts of immature mice. *Journal of Reproduction and Fertility*, **76**, 105–113.

Papaioannou, V.E., Gardner, R.L., McBurney, M.W., Babinet, C. and Grans, M.J. (1978) Participation of cultured teratocarcinoma cells in mouse embryogenesis. *Journal of Embryology and Experimental Morphology*, **44**, 93–104.

Parkes, A.S. (1924) On the migration of ova in the rabbit. *Journal of Physiology*, **59**, 357–360.

Pemble, L.B. and Kaye, P.L. (1986) Whole protein uptake and metabolism by mouse blastocysts. *Journal of Reproduction and Fertility*, **78**, 149–157.

Penketh, R.J.A., Delhanty, J.D.A., Van De Berghe, J.A., Finkelstone, E.M., Handyside, A.H., Malcolm, S. and Winston, R.M.L. (1989) Rapid sexing of human embryos by non-radioactive in situ hybridisation: potential for preimplantation diagnosis of X-linked disorders. *Prenantal Diagnosis*, **9**, 489–500.

Perry, J.S. and Rowlands, I.W. (1962) Early pregnancy in the pig. *Journal of Reproduction and Fertility*, **4**, 175–188.

Petters, R.M., Johnson, B.H., Reed, M.L. and Archibong, A.E. (1990) Glucose, glutamine and inorganic phosphate in early development of the pig embryo *in vitro*. *Journal of Reproduction and Fertility*, **89**, 269–275.

Pierce, G.B. and Dixon, F.J. (1959) Testicular tera-

tomas. II. Teratocarcinomas as an ascites tumor. *Cancer*, **12**, 584–589.

Platia, M.P. and Krudy, A.G. (1986) Transvaginal fluoroscopic recanalization of a proximally occluded oviduct. *Fertility and Sterility*, **44**, 704–706.

Polge, C. (1978) Embryo transfer and embryo preservation in *Artificial Breeding of Non-Domestic Animals*, (ed. P.F. Watson), Academic Press, London, pp. 303–316.

Prather, R.S. and First, N.L. (1990) Cloning embryos by nuclear transfer. *Journal of Reproduction and Fertility*, Suppl. 41, 125–134.

Pratt, H.P.M. and Muggleton-Harris, A.L. (1988) Cycling cytoplasmic factors that promote mitosis in the cultured 2-cell mouse embryos. *Development*, **104**, 115–120.

Pratt, H.P.M., Bolton, V.N. and Gudgeon, K.A. (1983) The legacy from the oocyte and its role in controlling early development of the mouse embryo in *Molecular Biology of Egg Maturation*, (Ciba Symposium 98), Alan R. Liss, New York, pp. 197–227.

Pursel, V.G., Hammer, R.E., Bolt, D.J., Palmiter, R.D. and Brinster, R.O. (1990) Integration, expression and germ-line transmission of growth-related genes in pigs. *Journal of Reproduction and Fertility*, **41**, 77–87.

Quinn, P. and Harlow, G.M. (1978) The effect of oxygen on the development of preimplantation mouse embryos *in vitro*. *Journal of Experimental Zoology*, **206**, 73–80.

Rall, W.F. and Fahy, G.M. (1985) Ice-free cryopreservation of mouse embryos at −196°C by vitrification. *Nature*, **313**, 573–575.

Rastan, S., Kaufman, M.H., Handyside, A.H. and Lyon, M.F. (1980) X-chromosome inactivation in extra-embryonic membranes of diploid parthenogenetic mouse embryos demonstrated by differential staining. *Nature*, **288**, 172–173.

Renfree, M.B. (1978) Embryonic diapause in mammals – a developmental strategy in *Mechanisms of Dormancy and Developmental Arrest*, (ed. M.E. Clutter), Academic Press, New York, pp. 1–46.

Renfree, M.B. (1982) Implantation and placentation in *Reproduction in Mammals*, 2nd edn, Book 2, *Embryonic and Fetal Development*, Chapter 2, (eds C.R. Austin and R.V. Short), Cambridge University Press.

Rexroad Jr., C.E., Hammer, R.E., Behringer, R.R., Palmiter, R.D. and Brinster, R.L. (1990) Insertion, expression and physiology of growth-regulating genes in ruminants. *Journal of Reproduction and Fertility*, Suppl. 41, 119–124.

Rhine, S.A. and Milunsky, A. (1979) Utilization of trophoblast for early prenatal diagnosis in *Genetic Disorders of the Fetus*, (ed. A. Milunsky), Plenum Press, New York, pp. 527–539.

Robertson, E.J. (ed.) (1987) *Teratocarcinomas and Embryonic Stem Cells: a Practical Approach*, IRL Press, Oxford.

Robinson, D.H., Smith, P.R. and Benos, D.J. (1990) Hexose transport in preimplantation rabbit blastocysts. *Journal of Reproduction and Fertility*, **89**, 1–11.

Roschlau, K., Rommel, P., Andreewa, L., Zackel, M., Roschlau, D., Zackel, B., Schwerin, M., Huhn, R. and Gazarjan, K.G. (1989) Gene transfer experiments in cattle. *Journal of Reproduction and Fertility*, Suppl. 38, 153–160.

Rossant, J. (1976) Postimplantation development of blastomeres isolated from 4- and 8-cell mouse eggs. *Journal of Embryology and Experimental Morphology*, **36**, 283–290.

Rossant, J. (1986) Development of extraembryonic cell lineages in the mouse embryo in *Experimental Approaches to Mammalian Embryonic Development*, (eds J. Rossant and R.A. Pedersen), Cambridge University Press, pp. 97–120.

Rossant, J. and Frels, W.I. (1980) Interspecific chimaeras in mammals: successful production of live chimaeras between *Mus musculus* and *Mus caroli*. *Science*, **208**, 419–421.

Rossant, J. and Frels, W.I. (1981) The origin of trophoblast and its role in implantation in *Cellular and Molecular Aspects of Implantation*, (eds S.R. Glasser and D.W. Bullock), Plenum Press, New York, pp. 43–54.

Rossant, J. and McBurney, M.W. (1982) The developmental potential of a euploid teratocarcinoma cell line after blastocyst injection. *Journal of Embryology and Experimental Morphology*, **70**, 99–112.

Runner, M.N. (1947) Development of mouse eggs in the anterior chamber of the eye. *Anatomical Record*, **98**, 1–47.

Ryan, J.P., O'Neill, C. and Wales, R.G. (1990a) Oxidative metabolism of energy substrates by preimplantation mouse embryos in the presence of platelet-activating factor. *Journal of Reproduction and Fertility*, **89**, 301–307.

Ryan, J.P., Spinks, N.R., O'Neill, C. and Wales, R.G. (1990b) Implantation potential and fetal viability of mouse embryos cultured in media

supplemented with platelet-activating factor. *Journal of Reproduction and Fertility*, **89**, 309–315.

Schnieke, A., Harbers, K. and Jaenisch, R. (1983) Embryonic lethal mutation in mice induced by retrovirus insertion into the alpha 1(I) collagen gene. *Nature*, **304**, 315–320.

Schultz, G.A. and Tucker, E.B. (1977) Protein synthesis and gene expression in preimplantation rabbit embryos in *Development in Mammas*, Vol. 2, (ed. M.H. Johnson), North-Holland, Amsterdam, pp. 69–97.

Seidel, F. (1952) Die Entwicklungspontenzen einer isolierten Blastomere des Zweizellenstadiums in Saugetierei. *Naturwissenschaften*, **39**, 355–356.

Seidel Jr., G.E., Elsden, R.P., Takeda, T. and Farrand, G.D. (1983) Field trials with cryopreserved bovine embryos in *Fertilization of the Human Egg In Vitro*, (eds H.M. Beier and H.R. Lindner), Springer-Verlag, Berlin, pp. 343–352.

Selwood, L. (1987) Embryonic development in culture of two dasyurid marsupials, *Sminthopsis crassicudata* (Gould) and *Sminthopsis macroura* (Spenser), during cleavage and blastocyst formation. *Gamete Research*, **16**, 355–370.

Selwood, L. and Young, G.J. (1983) Cleavage *in vivo* and in culture in the Dasyurid marsupial *Antechinus stuartii* (Macleay). *Journal of Morphology*, **176**, 43–60.

Sharman, G.B. (1955) Studies on marsupial reproduction. III. Normal and delayed pregnancy in *Setonyx brachyurus*. [Delayed birth in *Protemnodon eugenii*.] *Australian Journal of Zoology*, **3**, 56–70.

Shaw, J.M., Kola, I., MacFarlane, D.R. and Trounson, A.O. (1991) An association between chromosomal abnormalities in rapidly frozen 2-cell mouse embryos and the ice-forming properties of the cryoprotective solution. *Journal of Reproduction and Fertility*, **91**, 9–18.

Sherman, M.I. (1979) Developmental biochemistry of preimplantation mammalian embryos. *Annual Review of Biochemistry*, **48**, 443–470.

Smith, A.H. and Kleiber, M. (1950) Size and oxygen consumption in fertilized eggs. *Journal of Cellular Comparative Physiology*, **35**, 131–140.

Smith, A.U. (1961) *Biological Effects of Freezing and Supercooling*, Williams & Wilkins, Baltimore.

Smithies, O., Gregg, G., Boggs, B.S., Koralewski, M.A. and Kucherlapati, R.S. (1985) Insertion of DNA sequence into the chromosomal β-globin locus by homologous recombination. *Nature*, **317**, 230–234.

Solter, D. (1987) Inertia of the embryonic genome in mammals. *Trends in Genetics*, **3**, 23–27.

Soupart, P. (1980) Initiation of mouse embryonic development by oocyte fusion. *Archives of Andrology*, **5**, 55–57.

Soupart, P. (1982) *In Vitro Fertilization and Embryo Transfer*, (eds E.S.E. Hafez and K. Semm), MTP Press, Lancaster, pp. 51–63.

Spemann, H. (1938) *Embryonic Development and Induction*. Hafner Publishing Company, New York.

Spielmann, H. (1976) Embryo transfer technique and action of drugs on the preimplantation embryo in *Developmental Biology and Pathology*, (eds A. Gropp and K. Benirschke), Springer-Verlag, Berlin, pp. 87–103.

Steptoe, P.C. and Edwards. R.G. (1978) Birth after the reimplantation of a human embryo. *Lancet*, **ii**, 366.

Stern, M.S. (1973) Chimaeras obtained by aggregation of mouse eggs with rat eggs. *Nature*, **243**, 472–473.

Stern, S. and Biggers, J.D. (1968) Enzymic estimation of glycogen in the cleaving mouse embryo. *Journal of Experimental Zoology*, **168**, 61–66.

Stevens, L.C. (1958) Studies of transplantable testicular teratoma of strain 129 mice. *Journal of the National Cancer Institute*, **20**, 1257–1295.

Stevens, L.C. (1964) Experimental production of testicular teratomas in mice. *Proceedings of the National Academy of Sciences of the USA*, **52**, 654–661.

Stevens, L.C. (1970) The development of transplantable teratocarcinomas from intratesticular grafts of pre and postimplantation mouse embryos. *Developmental Biology*, **21**, 364–382.

Stevens, L.C. (1978) Totipotent cells of parthenogenetic origin in a chimaeric mouse. *Nature*, **276**, 266–267.

Stevens, L.C. (1981) Teratomas derived from germ cells and embryos in *Mechanisms of Sex Differentiation in Animals and Man*, (eds C.R. Austin and R.G. Edwards), Academic Press, London, pp. 301–328.

Stevens, L.C. and Varnum, D.S. (1974) The development of teratomas from parthenogentically activated ovarian mouse eggs. *Developmental Biology*, **37**, 369–380.

Stevens, L.C., Varnum, D.S. and Eicher, E.M. (1977) Viable chimaeras produced from normal and parthenogenetic mouse embryos. *Nature*, **269**, 515–517.

Stewart, C.L. (1982) Formation of viable chiaemras

by aggregation between teratocarcinomas and preimplantation mouse embryos. *Journal of Embryology and Experimental Morphology*, **67**, 167–179.

Stewart, T.A., Wagner, E.F. and Mintz, B. (1982) Human β-globin gene sequences injected into mouse eggs, retained in adults, and transmitted to progeny. *Science*, **217**, 1046–1048.

Storb, U., Arp, B. and Wilson, R. (1979) The switch region associated with immunoglobulin Cu genes is DNase hypersensitive in T lymphocytes. *Nature*, **294**, 90–92.

Sugawara, S. and Umezu, M. (1961) Studies on metabolism of the mammalian ova. II. Oxygen consumption of the cleaved ova of the rat. *Tohoku Journal of Agricultural Research*, **12**, 17.

Sugie, T. (1965) Successful transfer of a fertilized bovine egg by non-surgical techniques. *Journal of Reproduction and Fertility*, **10**, 197–201.

Summers, P.M., Shepherd, A.M., Hodges, J.K., Kydd, J., Boyle, M.S. and Allen, W.R. (1987) Successful transfer of the embryos of Przewalski's horses (*Equus przewalskii*) and Grant's zebra (*E. burchelli*) to domestic mares (*E. caballus*). *Journal of Reproduction and Fertility*, **80**, 13–20.

Suomalainen, E. (1950) Parthenogenesis in animals. *Advances in Genetics*, **3**, 193–253.

Surani, M.A.H. (1984) Differential roles of paternal and maternal genomes during embryogenesis in the mouse. *BioEssays*, **1**, 224–227.

Surani, M.A.H. (1991) Influence of genome imprinting on gene expression, pheotypic variations and development. *Human Reproduction*, **6**, 45-51.

Surani, M.A.H. and Barton, S.C. (1983) Development of gynogenetic eggs in the mouse; implications for parthenogenetic embryos. *Science*, **222**, 1034–1036.

Surani, M.A.H., Barton, S.C. and Kaufman, M.H. (1977) Development to term of chimaeras between diploid parthenogenetic and fertilized embryos. *Nature*, **270**, 601–602.

Surani, M.A.H., Barton, S.C. and Norris, M.L. (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature*, **308**, 548–550.

Surani, M.A.H., Barton, S.C. and Norris, M.L. (1986) Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome. *Cell* **45**, 127–136.

Surani, M.A.H., Barton, S.C. and Norris, M.L. (1987) Experimental reconstruction of mouse eggs and embryos: an analysis of mammalian development. *Biology of Reproduction*, **36**, 1–16.

Szollosi, D.G. (1965) The fate of sperm middlepiece mitochondria in the rat egg. *Journal of Experimental Zoology*, **158**, 367–377.

Szollosi, D.G. (1976) Oocyte maturation and the paternal contribution to the embryo in mammals in *Developmental Biology and Pathology*, (eds A. Gropp and K. Benirschke), Springer-Verlag, Berlin, pp. 9–27.

Tachi, C. and Tachi, S. (1980a) Developmental capacities of unfertilized mouse ova experimentally fused with polar bodies. *Zoological Magazine (Japan)*, **89**, 412.

Tachi, S. and Tachi, C. (1980b) Electron microscopic studies of chimeric blastocysts experimentally produced by aggregating blastomeres of rat and mouse embryos. *Developmental Biology*, **80**, 18–127.

Tachi, S., Tachi, C. and Knyszynski, A. (1985) Mechanisms underlying regulation of local immune responses in the uterus during early gestation of eutherian mammals. II. Electron microscopic studies on the interactions between blastocysts and macrophages cultured together *in vitro*. *Zoological Science*, **2**, 671–680.

Tadir, Y., Wright, W.H., Vafa, O., Liaw, L.H., Asch, R. and Berns, M.W. (1991) Micromanipulation of gametes using laser microbeams. *Human Reproduction*, **6**, 1011–1016.

Takagi, N. and Sasaki, M. (1975) Preferential inactivation of the paternally derived X-chromosome in the extra-embryonic membranes of the mouse. *Nature*, **256**, 640–641.

Talansky, B.E., Malter, H.E. and Cohen, J. (1991) A preferential site for sperm–egg fusion in mammals. *Molecular Reproduction and Development*, **28**, 183–188.

Tarkowski, A.K. (1959) Experiments on the development of isolated blastomeres of mouse eggs. *Nature*, **184**, 1286–1287.

Tarkowski, A.K. (1961) Mouse chimaeras developed from fused eggs. *Nature*, **190**, 857–860.

Tarkowski, A.K. (1963) Studies on mouse chimaeras developed from eggs fused *in vitro*. *National Cancer Institute Monographs*, **11**, 51–71.

Tarkowski, A.K. (1971) Recent studies on parthenogenesis. *Journal of Reproduction and Fertility*, Suppl. 14, pp. 31–39.

Tarkowski, A.K. and Wroblewska, J. (1967) Development of blastomeres of the mouse egg isolated at the 4- to 8-cell stage. *Journal of*

Embryology and Experimental Morphology, **18**, 155–180.

Tarkowski, A.K., Witowska, A. and Nowicka, J. (1970) Experimental parthenogenesis in the mouse. *Nature*, **226**, 162–165.

Thadani, V.M. (1979) Injection of sperm heads into immature rat oocytes. *Journal of Experimental Zoology*, **210**, 161–168.

Thadani, V.M. (1980) A study of heterospecific sperm–egg interactions in the rat, mouse and deer mouse using *in vitro* fertilisation and sperm injection. *Journal of Experimental Zoology*, **212**, 435–442.

Thomson, J.L. and Brinster, R.L. (1966) Glycogen content of preimplantation mouse embryos. *Anatomical Record*, **155**, 97–102.

Trounson, A. (1983) Comparative embryo transfer in Australia. *Theriogenology*, **19**, 17–29.

Trounson, A. and Mohr, L. (1983) Human pregnancy following cryopreservation, thawing and transfer of an 8-cell embryo. *Nature*, **305**, 707–709.

Tsunoda, Y., Tokunaga, T. and Sugie, T. (1985a) Altered sex ratio of live young after transfer of fast- and slow-developing mouse embryos. *Gamete Research*, **12**, 301–304.

Tsunoda, Y., Tokunaga, T., Sugie, T. and Katsumata, M. (1985b) Production of monozygotic twins following the transfer of bisected embryos in the goat. *Theriogenology*, **24**, 337–342.

Tucker, E.M., Moor, R.M. and Rowson, L.E.A. (1974) Tetraparental sheep chimaeras induced by blastomere transplantation. Changes in blood types with age. *Immunology*, **26**, 613–621.

Tucker, E.M., Dain, A.R. and Moor, R.M. (1978) Sex chromosome chimaerism and transmission of blood group genes by tetraparental rams. *Journal of Reproduction and Fertility*, **54**, 77–83.

Tyndale-Biscoe, H. (1973) *Life of Marsupials*, Arnold, London.

Tyndale-Biscoe, H. and Renfree, M. (1987) *Monographs on Marsupial Biology: Reproduction in Marsupials*, Cambridge University Press, Cambridge, UK.

Uehara, T. and Yanagamachi, R. (1976) Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. *Biology of Reproduction*, **15**, 467–472.

Usui, N. and Yanagamachi, R. (1976) Behaviour of hamster sperm nuclei incorporated into eggs at various stages of maturation, fertilization and early development. *Journal of Ultrastructural Research*, **57**, 276–288.

Van Blerkom, J. and Runner, M.N. (1976) The fine structural development of preimplantation mouse parthenotes. *Journal of Experimental Zoology*, **196**, 113–124.

Voekel, S.A., Humes, P.E. and Godke, R.A. (1984) Pregnancy rates resulting from non-surgical transfer of micromanipulated bovine embryos. *10th International Congress on Animal Reproduction and Artificial Insemination*, University of Illinois, Urbana-Champaign, USA, June 10–14, p. 251.

Wagner, E.F., Covarrubias, L., Stewart, T.A. and Mintz, B. (1983) Prenatal lethalities in mice homozygous for human growth hormone gene sequences integrated in the germ line. *Cell*, **35**, 647–655.

Wales, R.G. (1973) Biochemistry of the developing embryo. *Journal of Reproduction and Fertility*, Suppl. 18, pp. 117–125.

Wales, R.G. (1986) Measurement of metabolic turnover in single mouse embryos. *Journal of Reproduction and Fertility*, **76**, 717–725.

Wales, R.G. and Brinster, R.L. (1968) The uptake of hexoses by pre-implantation mouse embryos *in vitro*. *Journal of Reproducton and Fertility*, **15**, 415–422.

Ward, K.A., Murray, J.D., Nancarrow, C.D., Boland, M.P. and Sutton, R. (1984) The role of embryo gene transfer in sheep breeding programme in *Reproduction in Sheep* (eds D.R. Lindsay and D.T. Pearce), Australian Academy of Science and Australian Wool Corporation, Canberra, pp. 279–285.

Warr, J.R. (1984) *Genetic Engineering in Higher Organisms*, Institute of Biology's Studies in Biology, No. 162, Arnold, Baltimore.

Weatherall, D.J. (1985) *The New Genetics and Clinical Practice*, 2nd edn, Oxford University Press, Oxford, UK.

Weitlauf, H.M. (1974) Metabolic changes in the blastocysts of mice and rats during delayed implantation. *Journal of Reproduction and Fertility*, **39**, 213–224.

Weitlauf, H.M. (1976) Effect of uterine flushings on RNA synthesys by 'implanting' and 'delayed implanting' mouse blastocysts *in vitro*. *Biology of Reproduction*, **14**, 566–571.

Whitten, W.K. (1956) Culture of tubal mouse ova. *Nature*, **157**, 96.

Whitten, W.K. (1957) Culture of tubal ova. *Nature*, **179**, 1081–1082.

Whitten, W.K. (1971) Nutrient requirements for culture of preimplantation embryos *in vitro*. *Advances in the Biosciences*, **6**, 129–139.

Whitten, W.K. and Biggers, J.D. (1968) Complete development *in vitro* of the preimplantation stages of the mouse in a simple chemically defined medium. *Journal of Reproduction and Fertility*, **17**, 399–401.

Whitten, W.K. and Dagg, C.P. (1961) Influence of spermatozoa on the cleavage rate of mouse eggs. *Journal of Experimental Zoology*, **148**, 173–183.

Whittingham, D.G. (1968) Fertilization of mouse eggs *in vitro*. *Nature*, **220**, 592–593.

Whittingham, D.G. (1971) Survival of mouse embryos after freezing and thawing. *Nature*, **233**, 125–126.

Whittingham, D.G. (1974) Embryo banks in the future of developmental genetics. *Genetics*, **78**, 395–402.

Whittingham, D.G. (1975) Fertilization *in vitro* and development to term of unfertilized mouse oocytes previously stored at −196°C with DMSO in liquid N_2 in *Basic Aspects of the Freeze Preservation of Mouse Strains*, (ed O. Muhlbock), Gustav-Fischer Verlag, Stuttgart.

Whittingham, D.G. (1976) General aspects of egg culture and preservation in *Egg Transfer in Cattle* (ed. L.E.A. Rowson), Proceedings of the EEC Agricultural Research Seminar, Directorate General, Commision of European Communities, Luxembourg, pp. 101–113.

Whittingham, D.G. (1977) Fertilization *in vitro* and development to term of unfertilized mouse oocytes previously stored at −196°C. *Journal of Reproduction and Fertility*, **49**, 89–94.

Whittingham, D.G. (1980a) Principles of embryo preservation in *Low Temperature Preservation in Medicine and Biology*, (eds M.J. Ashwood-Smith and J. Farrant), Pitman Medical, London, pp. 65–83.

Whittingham, D.G. (1980b) Parthenogenesis in mammals in *Oxford Reviews of Reproductive Biology*, Vol. 2, (ed C.A. Finn), Oxford University Press, pp. 205–231.

Whittingham, D.G., Leibo, S.P. and Mazur, P. (1972) Survival of mouse embryos frozen to −196°C and −269°C. *Science*, **178**, 411–414.

Wiley, L.M. (1984) Cavitation in the mouse preimplantation embryo: Na^+/K^+-ATPase and the origin of nascent blastocoele fluid. *Developmental Biology*, **105**, 330–342.

Wiley, L.M., Yamani, S., and Van Muyden, D. (1986) Effect of potassium concentration, type of protein supplement and embryo density on mouse preimplantation development *in vitro*. *Fertility and Sterility*, **45**, 111–119.

Willadsen, S.M. (1979) A method for culture of micromanipulated sheep embryos and its use to produce monozygotic twins. *Nature*, **277**, 298–300.

Willadsen, S.M. (1980) The viability of early cleavage stages containing half the normal number of blastomeres in sheep. *Journal of Reproduction and Fertility*, **59**, 357–362.

Willadsen, S.M. (1981) The developmental capacity of blastomeres from four- and eight-cell sheep embryos. *Journal of Embryology and Experimental Morphology*, **65**, 165–172.

Willadsen, S.M. (1986) Nuclear transplantation in sheep embryos. *Nature*. **320**, 63–65.

Willadsen, S.M. and Fehilly, C.B. (1983) The developmental potential and regulatory capacity of blastomeres from two-, four- and eight-cell sheep embryos in *Fertilization of the Human Egg In Vitro*, (eds H.M. Beier and H.R. Lindner), Springer-Verlag, Berlin, pp. 353–357.

Willadsen, S.M., Polge, C., Rowson, L.E.A. and Moor, R.M. (1974) Preservation of sheep embryos in liquid nitrogen (abstract). *Cryobiology*, **11**, 560.

Willadsen, S.M., Trounson, A.O., Polge, C., Rowson, L.E.A. and Newcomb, R. (1976) Low temperature storage of cool eggs in *Egg Transfer in Cattle*, (ed. L.E.A. Rowson), Proceedings of the EEC Research Seminar, Directorate General, Commision of European Communities, Luxembourg, pp. 117–124.

Willadsen, S.M., Polge, C. and Rowson, L.E.A. (1978) The viability of deep frozen cow embryos. *Journal of Reproduction and Fertility*, **52**, 391–393.

Williamson, R., Eskdale, J., Coleman, D.V., Niazi, M. and Loeffley, F.E. (1981) Direct gene analysis of chorionic villi: a possible technique for first-trimester antenatal diagnosis of haemoglobinopathies. *Lancet*, **ii**, 1125–1127.

Wilmut, I. (1972) Effect of cooling rate, warming rate, cryoprotective agent and stage of development on survival of mouse embryos during cooling and thawing. *Life Sciences*, **11**, 1071–1079.

Wilmut, I. and Rowson, L.E.A. (1973) Experiments on the low temperature preservation of cow embryos. *Veterinary Record*, **92**, 686–690.

Wilson, E.B. (1928) *The Cell in Development and Heredity*, Macmillan, New York.

Wimsatt, W.A. (1975) Some comparative aspects of implantation. *Biology of Reproduction*, **12**, 1–40.

Wolf, D.E. (1983) The plasma membrane in early embryogenesis in *Development in Mammals*, Vol. 5, (ed. M.H. Johnson), Elsevier Science Publishers, Amsterdam, pp. 187–208.

Wright, J.M. (1985) Commercial freezing of bovine embryos in straws. *Theriogenology*, **23**, 17–29.

Yamada, K., Stevenson, A.F.G. and Mettler, L. (1988) Fertilization through spermatozoal microinjection: significance of acrosome reaction. *Human Reproduction*, **3**, 657–661.

Yamamoto, Y., Oguri, N., Tsutsumi, Y. and Hachinohe, Y. (1982) Experiments in the freezing and storage of equine embryos. *Journal of Reproduction and Fertility*, Suppl. 32, 399–403.

Yang, X., Chen, J., Chen, Y.Q. and Foote, R.H. (1990) Improved developmental potential of rabbit oocytes fertilized by sperm microinjection into the perivitelline space enlarged by hypertonic media. *Journal of Experimental Zoology*, **255**, 114–119.

Yotsuyanagi, Y. and Szollosi, D.G. (1980) Embryo development and intracisternal particles in the mouse. *Biologie Cellulaire*, **39**, 201–204.

Yotsuyanagi, Y. and Szollosi, D.G. (1981) Early mouse intracisternal particle: fourth type of retrovirus-like particle associated with the mouse. *Journal of the National Cancer Institute*, **67**, 677–685.

Yovich, J.L., Matson, P.L., Blackledge, D.G., Turner, S.R., Richardson, P.A. and Draper, R. (1987) Pregnancies following pronuclear stage tubal transfer. *Fertility and Sterility*, **48**, 451–457.

Zeilmaker, G. (1973) Fusion of rat and mouse morulae and formation of chimaeric blastocysts. *Nature*, **242**, 115–116.

Zinder, N. and Lederberg, J. (1952) Genetic exchange in *Salmonella*. *Journal of Bacteriology*, **64**, 679–699.

IMPLANTATION 3

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3.1 INTRODUCTION

In the two or three decades prior to the first edition of this book much of the basic morphological analysis of implantation and placentation took place so that Marshall (1910) was able to give a fairly detailed account of the early stages of placentation in several species. His account now provides a very interesting historical survey of the subject. Marshall did not separate implantation from placentation, in fact he only used the word implantation when talking of the implantation chamber which had been described by other workers. He mainly refers to attachment and embedding of the blastocyst.

The fact that embedding was accompanied by decidualization and only occurred in some species had been known for some time (section 3.4.5). Huxley (1869) divided species into deciduate and non-deciduate.

Nowadays the word embedding has been largely replaced by implantation. However, currently implantation usually refers to the initial stage of placentation in all species regardless of whether the blastocyst is embedded in the wall of the uterus or remains in the lumen. The word nidation is sometimes used, usually with regard to those species which undergo decidualization. The large decidual cells are likened to a nest or nidus. The use of this word implies that the uterine response is a passive protective structure, whereas the cells are now known to be actively secretory (section 3.5.4).

3.2 SPECIES VARIATION

In evolutionary terms the attachment of the ovum to the wall of the uterus is a fairly recent event. Sexual reproduction started with the joining of gametes in the external environment and has gradually evolved through an intermediate stage in which there is internal fertilization of the ovum with external development of the embryo, to the final stage where the embryo develops inside the wall of the uterus. The evolution of viviparity can be traced through non-mammalian species (Amoroso, 1981; Heyner, 1981), but it is in mammals that implantation has achieved its most complex form.

It is difficult, especially in the simplest forms of attachment between the mother and offspring, where there is little uterine reaction to the blastocyst, to separate implantation from the later stages of placentation. In order to avoid too much overlap, detailed descriptions of the early stages of placentation in species without much uterine response will be dealt with in Chapter 4 on placentation.

The simplest form of viviparity is where the embryo develops inside the mother but, isolated from her by a membrane, uses nutrients contained within the egg. The next stage is the development of the egg inside the mother but utilizing nutrients supplied by the mother, mainly by glandular secretions. In this there is no attachment of the fetal membranes and it is doubtful if the term implantation should be applied.

The simplest form of implantation is where the trophoblast of the embryo becomes closely applied to the surface of maternal cells so that nutrients can pass from the mother to the embryo directly across cell membranes. Among domestic animals this is found in the pig (Perry and Rowlands, 1962) and horse (Amoroso, 1952). In this form of implantation nutrients have to pass through several tissues from the maternal bloodstream to the embryo. To compensate for this the blastocyst, or at least the non-embryonic part of it, expands massively before implantation, so that the area of contact between mother and fetus increases dramatically. At the same time the surface of the trophoblast cells develops microvilli which interdigitate with similar microvilli on the luminal surface of the uterine epithelial cells. The result is that a very large area of close contact between maternal and fetal

tissues is established to produce a very efficient organ of exchange. A newborn foal is testimony to this.

Once this stage has been reached, the blastocyst cannot be easily washed from the uterus and the embryo is said to be attached to the uterine lumen. It is thought that the close contact between trophoblast and luminal epithelial surface is an active process brought about by changes in the cell surfaces (discussed in section 3.4.5). Most implanting blastocysts go through this stage which is called the 'attachment phase'. Enders and Schlafke (1967) distinguish it from the apposition stage which precedes it and during which the blastocyst can be easily removed from the uterus, implying lack of adhesive forces.

In spite of the effectiveness of the simple placenta, changes have taken place during evolution to bring the fetal and maternal blood system into closer contact, and one of the most fascinating facets of implantation is the variety of methods developed to ensure this (Finn, 1980). It is not proposed to give details of all the variations among species, as there are already several detailed accounts (Marshall, 1910; Amoroso, 1952; Boyd and Hamilton, 1952; Wimsatt, 1975), but it may be useful to highlight the main lines of development.

As long ago as 1882, Bonnet divided implantation into central, eccentric and interstitial. These terms are still used today. They refer, of course, to the position of the blastocyst in the uterus. In the first, the blastocyst remains in the centre of the uterine lumen and usually expands considerably just before implantation. In eccentric implantation the blastocyst remains small and comes to lie at one side of the uterine lumen as seen in transverse section, whilst in interstitial implantation the blastocyst burrows through the luminal epithelial cells.

Once settled in position the blastocyst and endometrium adopt various stratagems to 'improve' intimacy between fetal and maternal tissues. In the cow the uterine epithelial cells are initially columnar in shape. At implantation binucleate cells from the trophoblast pass into the uterine epithelium where they fuse with the columnar cells to form giant cells (Wooding and Wathes, 1980). For a short period most of the uterine epithelium consists of these giant cells. Subsequently, these cells die off and the remaining uterine columnar epithelial cells proliferate so that the maternal luminal epithelium is re-established (Wathes and Wooding, 1980).

Similarly, in the goat multinucleate cells are formed in the uterine epithelium, although there is, at present, no evidence that cells from the trophoblast contribute to these. These cells form a syncytium which later degenerates and is replaced by uterine columnar epithelial cells (Dent, 1973). In the ewe, like the cow, a syncytium replaces the columnar uterine epithelial cells (Boshier, 1969; Guillomot *et al.*, 1981; Wooding *et al.*, 1982), but this appears to be largely derived from the trophoblastic binucleate cells. Unlike in the cow and goat however, this syncytium is continuously replaced from binucleate trophoblast cells and remains throughout pregnancy.

These examples from domestic ungulates demonstrate the tendency of the uterine epithelium firstly to form a syncytium and then to degenerate, although in each case the epithelial layer is re-established either from the uterine epithelial cells or from trophoblast cells. It has been suggested that the purpose of this manoeuvre is to transfer a prolactin-like substance in the trophoblast binucleate cells into the uterine epithelium. The importance to the blastocyst of ensuring that the secretion of progesterone is maintained will be discussed in section 3.4.3.

In other species the uterine epithelium undergoes degeneration without subsequent re-epithelization. However, the mechanisms evolved to achieve this differ between species. For example, the rabbit undergoes a

process whereby, during the preparation of the uterus for implantation, the uterine epithelium becomes a syncytium whilst a similar reaction occurs in the trophoblast. At the time of implantation the two syncytia (maternal and fetal) fuse to form a single tissue originally containing nuclei from both animals. Eventually the maternal nuclei disappear so that the syncytium can now be considered fetal. This very novel way of getting rid of the maternal epithelium has only so far been reported in the rabbit (Larsen, 1970; Steer, 1970, 1971). In other animals it appears that the epithelial cells die by a process of programmed cell death in response to the presence of the trophoblast in contact with the uterine epithelial cells (discussed in section 3.4.6).

It appears that in evolution the ability of cells to undergo self-destruction in a controlled manner in response to the turning on of information contained in the nucleus [a process commonly used in embryonic modelling (Saunders, 1966)] has been adapted to allow the more intimate contact of the embryo with its mother.

However, not all species have gone in this direction in which, at least initially, the uterus is controlling the entry of the blastocyst into the uterine tissue. It was mentioned earlier that in the ungulates which have been studied cells migrate from the trophoblast into the uterine epithelium. It should also be added that the horse conceptus, later in pregnancy, sends chorionic cells into the substance of the uterus (W.R. Allen *et al.*, 1973). In these cases it appears that the object is to get large molecular weight hormones from the embryo to the mother. This is one of the first signs of the fetus endeavouring to influence its own destiny. Cell migration is carried a stage further in women and some other animals (chimpanzee, Heuser, 1940; guinea pig, Samson and Hill, 1931): it is not just isolated cells which migrate – large areas of trophoblast insinuate between the uterine epithelial cells. In order to do this some of the trophoblast cells form into a syncytium. Enders and Schlafke (1971) consider that this improves the flow characteristics of the tissue. It would presumably decrease the likelihood of the tissue breaking up during migration and preserve the organization of the blastocyst. The process is very rapid and the embryo becomes established in the uterine tissues with little apparent degeneration of the uterine epithelium. In the case of the human, and probably other species, the chorion does produce hormones, however the chief aim of trophoblast migration is not the passage of these to the mother; in fact it seems probable that chorionic gonadotrophins can reach the maternal circulation before the blastocyst has passed into the uterine tissues.

It is clear that the human embryo takes a much more active role in implantation than, for example, the pig or horse. However, the final product, the newborn animal, does not appear to be developmentally inferior in those species with simple placenta, and it is interesting to enquire what evolutionary advantage has come from the deep implantation of the blastocyst into the wall of the uterus, especially in view of the immunological and other problems which have had to be overcome. It is difficult to find any pattern relating the degree of intimacy in the attachment of the blastocyst with taxonomic position or size. Among the primates, for instance, there are species like man in which the ovum is deeply embedded in the wall of the uterus, whilst in other species, such as the marmoset, implantation is much more superficial.

With the blastocyst remaining small and coming into closer contact with the maternal blood vessels it is presumably possible to get adequate fetal nutrition with the involvement of a smaller area of uterus. This might be thought to be a special advantage to polytocous species. However, the pig, which has large numbers of offspring, implants superficially and gets round the problem by having a

very large uterus. Similarly, man, a species certainly larger than many which implant superficially, implants interstitially.

One possible advantage to an animal of attaining a very close contact between maternal and fetal bloodstreams is that it allows easier transfer of antibodies from the mother to the fetus, thus conferring on the neonate a degree of ready-made immunity which has to be acquired post-natally through the colostrum in those animals with simple implantation. This occasionally leads to problems, for example, when rhesus negative mothers are carrying rhesus-positive offspring. Nevertheless, overall it is probably advantageous to the species.

A great deal of care is needed in extrapolating information about implantation from one species to another. Owing to their ready availability a great deal of the research on implantation has been carried out on rats and mice, and these species have been particularly useful in providing basic information of the cell biology of the uterus and blastocyst. While much of this information will be of general applicability some will not. This is especially important when extrapolating from experimental animals to man (Finn, 1983).

3.2.1 DELAYED IMPLANTATION

Apart from differences in their method of implantation, species vary in the timing of implantation. In all animals the fertilized ovum appears to spend a defined period in the oviduct, reaching the uterus after having completed the early stages of development, usually as far as the blastocyst stage. There is then a variable period of free life in the uterus before attachment starts. Usually this period is short. In some species however the period can be varied in such a way as to give a measure of control over the time of birth of the offspring. The most common method of controlling the season of birth is by regulating the incidence of the oestrous cycle and ovulation in response to changes in the external environment. An alternative method is to insert a period of delay between the arrival of the blastocyst in the uterus and its implantation. This process of delayed implantation has evolved in several species over several taxonomic groups. In many cases the delay is inserted to allow a variable gestation period so that the young are born when the external environment is clement (roe deer, Ziegler, 1843; Aitken, 1974; badger, Canivenc and Bonnin-Laffargue, 1963; red kangaroo, M.J. Clark, 1968; mink, Hansson, 1947; Concannon *et al.*, 1980; black bear, Wimsatt, 1963; spotted skunk, Enders *et al.*, 1986). In these cases the delay is obligatory and controlled by seasonal factors in the environment (Mead, 1968; Hinds, 1989), probably via the pineal gland (May and Mead, 1986).

An alternative form of delayed implantation is related to the reproductive needs of the mother. This was first demonstrated in mice (Lataste, 1891; Kirkham, 1916). In this facultative form of delayed implantation, blastocysts are held quiescent in the uterus so as to delay the time of parturition of the litter until the mother has finished weaning the previous litter. This is common in marsupials (see Chapter 8) and rodents (rats, mice, bank vole, Andersson and Gustafsson, 1979).

Of course if implantation is delayed then development of the embryo must be halted, or slowed down very considerably. This condition of embryonic diapause is discussed here and in Chapters 6 and 8.

3.3 PREPARATION OF THE UTERUS FOR IMPLANTATION

3.3.1 THE OESTROUS CYCLE

The oestrous cycle, although referring semantically to behavioural events, covers a whole cycle of changes in the hypothalamus, pituitary, ovary and female reproductive tract. The transport of the ovum through the oviduct has to be precisely timed so that it

arrives in the uterus at the correct stage of development and at a time when the uterus is in a fit condition to receive it. The cellular and biochemical composition of the endometrium varies according to the hormonal influences acting on it and, although it has evolved to contain the blastocyst, it is, under most hormonal conditions, hostile to it; more hostile in fact than some other areas of the body. For example, if a blastocyst is transferred to the kidney or testis it will grow and destroy maternal tissue. In the uterus however, if the hormonal conditions are not appropriate, it will not survive and will itself be destroyed. It seems that the lining epithelium of the uterine epithelium under most conditions is resistant to attachment and invasion by the trophoblast and only under very precise hormonal states can implantation and survival of blastocysts take place.

In most animals the uterine conditions necessary for reception of the blastocyst develop during the later part of the oestrous cycle and receptivity of the uterus is controlled by the sequential secretion of ovarian hormones. As the same hormones control the passage of the ova along the oviduct, the coincidence of the arrival of the blastocyst in the uterine lumen with receptivity of the endometrium is assured.

A few animals, for example rats and mice, do not have a full oestrous cycle unless mating has occurred. Unmated animals, therefore, do not maintain the corpus luteum over the period during which ovum transport and implantation would be taking place. By abbreviating their oestrous cycles these animals, who are mostly solitary scavengers, maximize their chances of becoming pregnant. If a female is mated but the mating is infertile then the corpus luteum is maintained for about 11 days and a cycle with a luteal phase is attained. This so-called pseudopregnancy or extended oestrous cycle is equivalent to the normal oestrous cycle of most other animals. It is possible to extend the oestrous cycle in rats and mice without pregnancy by mating with a vasectomized male or in rats by artificially stimulating the vagina and cervix. In order to distinguish this from the abbreviated oestrous cycle it will be referred to as the 'extended' or 'mated' oestrous cycle and where general reference to oestrous cycles is made it will refer to cycles with a full luteal phase. The use of the term 'pseudopregnancy' is best reserved for a clinical condition in carnivores in which the bitch shows signs and symptoms of pregnancy.

During the course of the oestrous cycle the uterus is required to perform two functions. After copulation it provides the tube through which sperm must pass and in which it is capacitated before reaching the oviduct, there to fertilize the ovum. Later, after arrival of the blastocyst in the uterine lumen it provides the tissue in which implantation takes place. These two functions require different environmental conditions in the uterine lumen. For sperm capacitation and transport the uterine lumen is dilated, to a lesser or greater extent, with a thin watery fluid. Following fertilization, excess spermatozoa are removed, which probably involves the participation of eosinophil leucocytes. After arrival of the blastocyst in the lumen, the uterus is adapted, first to provide secretions for blastocyst nutrition and then to allow attachment and implantation of the trophoblast. The former involves differentiation of the uterine glands, and the latter differentiation of the luminal epithelium and stroma.

The cellular and biochemical changes that take place in the endometrium throughout the oestrous cycle have been most carefully worked out in rodents. This account therefore will be largely based on work on the rat and mouse, but, wherever possible, reference will be given to findings in other species.

The endometrium is made up of three main tissues – luminal epithelium, glandular epithelium and stroma (Figure 3.1).

Each of these has a specific role to play in

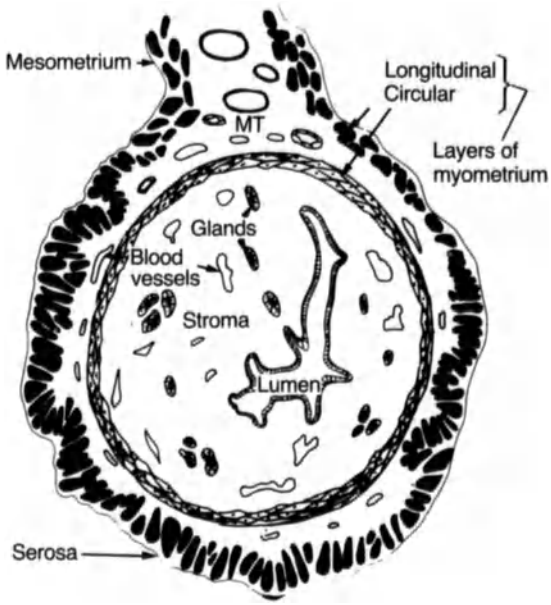

Figure 3.1 Diagrammatic cross-section of a uterus showing the main tissues (based on the mouse uterus).

implantation and each goes through a cycle of changes in preparation for this role. In addition to the resident cells making up the main tissues of the endometrium there are also cells which infiltrate from the bloodstream. Eosinophil leucocytes have already been mentioned, and during decidualization others become very important.

Each of the resident cell types undergoes three basic processes – cell proliferation, cell differentiation and cell death. Quantification of these processes is difficult although attempts have been made, especially with the first and last.

3.3.2 CELL PROLIFERATION

Cells which are undergoing mitosis can be seen in histological sections with the light microscope after normal staining. The condensation of the chromosomes during prophase and metaphase clearly distinguishes dividing from non-dividing cells. However, this stage of cell division is accomplished quickly so that even in an actively dividing tissue only a few cells will be visible. If, however, a drug is administered which destroys the activity of the microtubules, for example colchicine or vinblastine, then mitosis is halted in the metaphase stage. The animals can then be killed at a specific interval after the administration of the drug and all cells which have entered division during that period will be visible and can be counted. When dealing with the uterus it would be exceedingly tedious to completely section and count the whole organ, and normally a section is taken from each uterus of a group of animals and the mitoses counted in each tissue. Thus an estimate of the mean number of cells in mitosis is obtained with an estimation of the variability between animals. This provides useful data for comparisons between animals in various reproductive states or under different hormone regimens.

The results from such observations in the mouse (Finn and Martin, 1967) are demonstrated in Figure 3.2.

Cell division occurs at different times in the

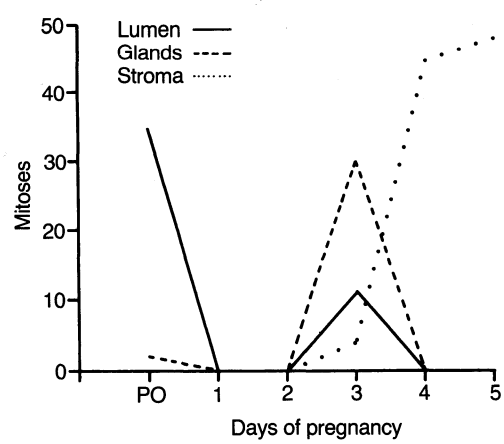

Figure 3.2 Graph showing the distribution of mitoses in the various tissues of the mouse uterus during early pregnancy. The animals were treated with colchicine 2 h before autopsy and the number of mitotic figures counted in one cross-section taken at random from each uterus. (Data from Finn and Martin, 1967.)

three uterine tissues. Luminal epithelial cells show maximum cell division at pro-oestrus with a second smaller increase on day 3 of pregnancy of the extended cycle. Glandular epithelial cells show a single peak of maximum cell division on day 3, whilst the stromal cells do not divide until day 4. There is thus an abrupt change in the pattern of cell division between days 3 and 4 of the mated cycle. A similar pattern has been shown in the rat (Leroy and Galand, 1969; Marcus, 1974a), guinea pig (Schmidt, 1943; Mehrotra and Finn, 1974; Marcus, 1974b) and hamster (Krueger and Craig-Maibenco, 1972). The situation in primates is not so well established. Human data come from biopsy material in which mitoses have not been pharmacologically arrested (Noyes *et al.*, 1950). It is difficult in this material to separate luminal from glandular cells. Nevertheless there appears to be a peak in epithelial mitosis during the follicular phase of the cycle with a peak in stromal mitosis in the luteal phase. Rhesus monkeys similarly show a peak in epithelial mitosis in the follicular phase (Bensley, 1951), but unfortunately no information seems to be available about stromal mitosis.

The animals for which information on cell division is available are those in which blastocysts pass into the endometrial stroma and the stroma decidualizes. It would be very interesting to know whether animals with superficial implantation, such as the pig, show stromal mitosis during the luteal phase. The situation with regard to mitosis in the rabbit, which, as mentioned earlier, has a very unusual method of blastocyst attachment, is unusual. There is a peak of mitosis on days 3 and 4 of pregnancy in the epithelial cells with very little mitosis in the stroma on any day up to day 10 (A.E. Lee and Dukelow, 1972; Davies and Hoffman, 1973).

An alternative method for assessing the rate of cell proliferation in tissues is to inject labelled thymidine into the animals at a determined interval before autopsy. All cells going into the S phase of the cell cycle will take up the nucleotide and they can be identified and counted in histological sections as before. On the whole, similar results have been obtained using this method as using colchicine (Leroy and Galand, 1969; Leroy *et al.*, 1969; Carter and McLaren, 1975). Another more recently developed method is to show the presence of proliferating nuclear antigen. Again, similar distribution of mitoses has been shown (Goodger and Rogers, 1993).

3.3.3 THE HORMONAL CONTROL OF PROLIFERATION

It has been known for many years that ovarian hormones influence cell division in the uterus (E. Allen *et al.*, 1937; Lloyd, 1937). E. Allen and his colleagues (1943) seem to have been the first to introduce the use of colchicine for the study of cell division in the uterus. During the following 30 years the effect of oestrogens, in particular, on the endometrial epithelium was confirmed (Perrota, 1962; Bertalanffy and Lau, 1963; Everett, 1963; Epifanova, 1966; Beato *et al.*, 1968; Leroy *et al.*, 1969). Most of the work was carried out on rats and mice, in which it was shown that oestrogen caused mitosis predominantly in the luminal epithelium. Work on rabbits demonstrated that progesterone stimulated proliferation of the glands (McPhail, 1934; Lloyd, 1937; A.E. Lee and Dukelow, 1972), whereas in the rat and mouse progesterone given alone had little effect on endometrial mitosis (luminal or glandular).

What was not apparent from the earlier work was the cause of change from predominantly luminal to predominantly glandular mitosis and then the abrupt change from epithelial to stromal during the extended oestrous cycle or early pregnancy in the rat and mouse. The answers became apparent when the effect of the ovarian hormones was studied, taking into account the fact that hormone secretion from the ovary occurs in a charac-

teristic temporal pattern. During the oestrous cycle there is secretion of high levels of oestradiol from the follicle during pro-oestrus followed by secretion of progesterone from the corpus luteum after ovulation. There is also in mice and rats a second small surge of oestrogen during the luteal phase of the cycle.

Using ovariectomized mice treated with exogenous ovarian hormones, Martin and Finn (1968) showed that the mitotic response of the endometrium to oestradiol was more complicated than originally thought. They demonstrated that the injection of a single dose of 100 ng of oestradiol into ovariectomized mice was followed 24 h later by division of the cells of the luminal epithelium. When daily injections were given and groups of animals killed every 24 h the luminal epithelial cells divided in response to the injections on the first 2 days but not to subsequent injections. However, animals killed after three daily injections showed large numbers of cells undergoing mitosis in the glandular epithelium (Finn and Martin, 1973). This rather surprising finding showed that the luminal and glandular epithelia respond differently to oestradiol. However, these results did not explain the rise in glandular mitosis on day 3 of the mated cycle. Oestrogen secretion is not high for longer than 2 days in late dioestrus and pro-oestrus in the mouse so continuous high levels of oestrogen could not explain the pattern of cell division during the oestrous cycle. Subsequent experiments showed that a differential effect on epithelial mitosis could be demonstrated if animals were given injections of 100 ng of oestradiol on only 2 days and killed at daily intervals for the following 5 days. The mitotic counts showed clearly that a level of oestradiol such as would be expected at pro-oestrus would, as before, have an immediate effect on mitosis in the luminal epithelium but a delayed one on the glandular, thus mimicking the situation observed during the mated cycle.

The next question concerns the cause of the switch from epithelial to stromal mitosis. Cell division is not seen in the endometrial stroma in the mouse in response to oestradiol. In view of the known presence of progesterone during the luteal phase it seemed likely that progesterone would be responsible for the switch from epithelial to stromal division between day 3 and day 4 of the mated cycle. However, the injection of progesterone into ovariectomized mice did not cause stromal mitosis, in fact after a single injection of the hormone cells undergoing division were found in the luminal epithelium. This unexpected result was explained when progesterone treatment was preceded by oestrogen, on a schedule to mimic the hormonal conditions of the mated cycle. Given at the appropriate time after oestrogen priming, a single injection of progesterone does stimulate stromal division. Combined with the previous results it was thus possible to reproduce the pattern of division characteristic of the mated cycle or early pregnancy by treating ovariectomized mice with two daily doses of 100 ng of oestradiol followed by 3 days rest then daily injections of 500 μg of progesterone. The numbers of stromal mitoses were not as high as expected. However, if a small dose of oestradiol is given in addition to the progesterone then a larger response is obtained. In fact, it is possible to get stromal proliferation without pro-oestrous oestrogen if progesterone is given for 3 or 4 days and a small quantity of oestradiol is added to the progesterone injections. Similar results have been obtained in rats (B.F. Clark, 1971). Recently the role of progesterone in inducing the switch from epithelial to stromal division has been confirmed using the antiprogestin, RU 486 (Cullingford and Pollard, 1988) and antibodies to progesterone (Heap *et al.*, 1988).

It is pertinent to ask what is the function of the cell division. As mentioned earlier, each uterine tissue, in preparation for implantation, differentiates to perform a specific function. It has been suggested that before

they can differentiate cells must perform a quantal mitosis, which in some way prepares them for differentiation. This was originally suggested for the mammary gland (Stockdale and Topper, 1966), but no concrete evidence has yet been found for such a role in the uterus.

An alternative, simpler explanation is that there is a need for an increased number of cells. Certainly the lumen has to increase very rapidly at oestrus, and increased cell division would be necessary. The glands, although not appearing to be as active in the mouse as in some animals, e.g. rabbit and man, presumably are brought up to full size by the cell division, whilst the number of stromal cells would need to increase to provide a stock of cells for decidualization.

3.3.4 INTRACELLULAR MECHANISMS IN CELL PROLIFERATION

Following the discovery of the importance of oncogenes and growth factors in cell proliferation, a considerable amount of effort has been expended in demonstrating their presence in the endometrium during pregnancy and after hormone treatment (Brigstock *et al.*, 1989; J.W. Pollard, 1990; Brigstock, 1991; Simmen and Simmen, 1991; Stancel *et al.*, 1993).

It appears that when oestradiol passes into a cell and attaches to its receptor (which is a transcription factor) several oncogenes are activated. The products of the nuclear oncogenes, c-*jun* and c-*fos* (which make up the transcription factor AP-1) increase within 2 h of oestradiol injection (Loose-Mitchell *et al.*, 1988; Weisz and Bresciani, 1988; Weisz *et al.*, 1990; P.J. Baker *et al.*, 1992). These genes are known as immediate early nuclear oncogenes. The presence of their products is short-lived and can be induced by oestrogenic compounds which are not able to initiate DNA synthesis and cell division (Persico *et al.*, 1990), indicating that some other factor is necessary for the completion of mitosis. This correlates well with the earlier finding that impeded oestrogens are not firmly bound in the nucleus and are not therefore able to induce an oestrogenic response (Martin, 1964). Progesterone inhibits their expression in the luminal epithelium (Kirkland *et al.*, 1992), which is consistent with the earlier finding that progesterone inhibits cell division in the luminal epithelium in response to oestradiol.

Other nuclear oncogene products found in the endometrium after oestrogen treatment include those from c-*myc* (Weisz and Bresciani, 1988; L.J. Murphy, 1991) and c-*ras* (Cheng and Pollard, 1986). The latter is analogous to a G protein and presumably plays an important role in the transmission of messages associated with mitosis.

Some of the products of other oncogenes stimulated by oestradiol are growth factors or growth factor receptors, for example that of c-*erb*-B is the receptor for both epidermal growth factor and transforming growth factor and its level is influenced by oestradiol (Lingham *et al.*, 1988). The product of c-*sis* is platelet-derived growth factor (see review Stancel *et al.*, 1993). Growth factors and their receptors play an important part in the reactions between the arrival of oestradiol in the cell and mitosis. Several have been demonstrated in the uterus. In most cases they are little in evidence in the uterus of the ovariectomized animal but increase after oestradiol treatment although basic fibroblast growth factor appears to be an exception to this (Wordinger *et al.*, 1992). In some cases their distribution is modified by progesterone in such a manner as would be expected from the hormonal control of mitosis. Growth factors and their receptors which are associated with hormone-induced mitosis in the uterus include epidermal growth factor (Mukku and Stancel, 1985a,b; DiAugustine *et al.*, 1988; Lin *et al.*, 1988; Huet-Hudson *et al.*, 1990), insulin-like growth factor (L.J. Murphy *et al.*, 1987; L.J. Murphy, 1991; Norstedt *et al.*, 1989; Kapur *et al.*, 1992), fibroblast growth factor

(Brigstock, 1988), platelet-derived growth factor (Stiles, 1985) and transforming growth factor (Tamada *et al.*, 1991). Other growth factors have been demonstrated in the uterus (colony stimulating factor 1, Bartocci *et al.*, 1986; transforming growth factor β Lea *et al.*, 1992) but their relationship with mitosis is less certain. They will be discussed in section 3.4.4 on cytokines and implantation.

The presence of growth factors and their receptors is by no means exclusive to the uterus. They are probably found in all cells which undergo division. The difference between endometrial cells and non-reproductive cells, such as those in the liver and skin, is that the prime stimulus for their synthesis comes from the ovary, whereas non-reproductive tissues are stimulated by other factors such as injury to the skin or removal of part of the liver. However, even in the uterus the presence of a blastocyst or a drop of oil or injury considerably increases cell division (section 3.5.3).

The intracellular mechanisms within the endometrial cells which bring about mitosis are similar to those within other cells and these have been studied in more detail, especially in cultured cells. The literature is very extensive but a good summary, relevant to reproduction, can be found in the review by Paton and Collins (1992) on the differentiation of ovarian granulosa cells.

Figure 3.3 Cross-section of mouse uterus under the influence of an oestrogen, treated to demonstrate the presence of alkaline phosphatase. (From Finn, 1971, by permission of the editor, *Advances in Reproductive Physiology*.)

3.3.5 CELL DIFFERENTIATION

(a) Luminal epithelium

In all animals the luminal surface of the epithelial lining to the uterus provides the first area of contact with the trophoblast. With the possible exception of animals undergoing interstitial implantation, the first stage of implantation involves adherence of the trophoblast to the luminal epithelium with very close contact between apposing surfaces. To attain this the surface of the epithelial cells has to undergo specialized changes. In many animals the changes can be seen at the appropriate stage of the extended oestrous cycle between apposing surfaces of the lumen even though blastocysts are not present. The changes can be followed in histological sections of uteri of animals killed at intervals during the cycle but for a full demonstration of the changes it is necessary to use the electron microscope.

After the burst of cell division, just before oestrus, the uterine lumen is dilated and irregular in cross-section; the epithelial cells are tall columnar with basophilic cytoplasm. Their luminal surface is active for alkaline phosphatase (Figure 3.3) (Arzac and Blanchet, 1948) and with the electron microscope can be shown to have numerous long microvilli, covered with a fuzzy material (glycocalyx).

During the first few days of the mated cycle the lumen is gradually reduced in size. This appears to be at least partly brought about by the death of many of the epithelial cells which follows the cessation of oestrogen action (section 3.3.7). With the formation of the corpus luteum a further loss of luminal fluid takes place by pinocytosis (Vokaer, 1952; Vokaer and Leroy, 1962; Enders and Nelson, 1973; M.B. Parr, 1980). In cross-section the uterine lumen now appears as a slit orientated in a mesometrial/antimesometrial axis (Figure 3.4).

The uptake of fluid from the lumen is accompanied by changes on the luminal surface of the epithelial cells. These can be seen when the cells are examined with the transmission electron microscope. Small blebs appear on the surface which have been shown to be absorbatent. With the scanning electron microscope they appear as 'small anemone-shaped structures' (Psychoyos and Mandon, 1971; Nilsson, 1972). These have been demonstrated in the uteri of rats (Enders and Nelson, 1973; M.B. Parr and Parr, 1974), ewes, cows (Guillomot and Guay, 1982) and women (Martel *et al.*, 1987). Endocytosis is probably also carried out by small coated vesicles (M.B. Parr and Parr, 1974, 1977). The cells are now regularly aligned and lipid droplets appear in the base of the cells (Alden, 1947). The tight junctions at the apical margins of the cells also change as differentiation proceeds (C.R. Murphy *et al.*, 1982a,b; Johnson *et al.*, 1988).

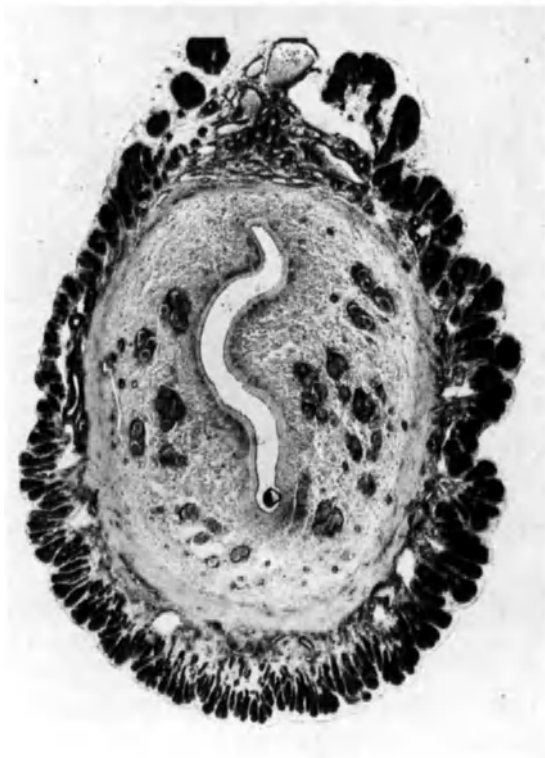

Figure 3.4 Cross-section of mouse uterus under the influence of progesterone. The lumen is slit shaped and orientated in the antimesometrial–mesometrial axis. The section is treated to demonstrate alkaline phosphatase which is present especially in the longitudinal muscle layer and the blastocyst. (From Finn and McLaren, 1967, by permission of the editor, *Journal of Reproduction and Fertility*.)

As a result of these and possibly other changes, the uterine lumen becomes almost obliterated with close interdigitation of the microvilli from apposing surfaces. This occurs at the time when the blastocysts have settled down to their determined position in the uterus and holds them firmly in place. It is called the first stage of closure (Figure 3.5) or the apposition stage of pregnancy.

With most fixatives used in light microscopy the apposing surfaces are pulled apart and have a scalloped or corrugated appearance in cross-section (Finn and McLaren, 1967). However, fixatives used for electron microscopy (glutaraldehyde) retain the closure (Nilsson, 1966).

During delayed implantation the uterus remains in this stage, but when implantation has been stimulated (section 3.4.3) a further change takes place in the lumen. On day 5 of the extended cycle in mice, the day on which implantation would be expected, the uterine lumen closes completely, so that the apposing surfaces now run together in a haphazard way with a small gap (about 150Å) between the apposing surfaces, 'the second stage of

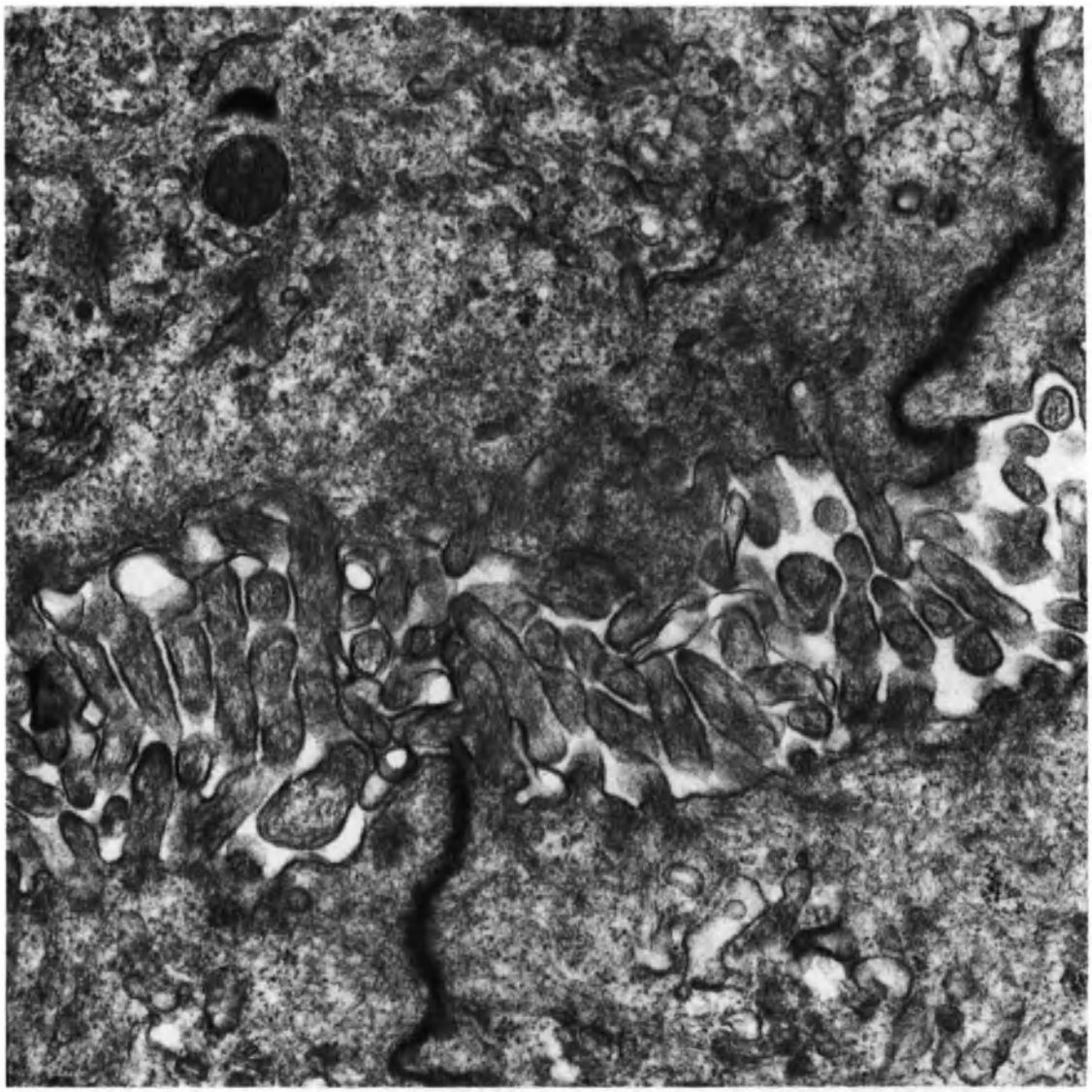

Figure 3.5 Electron micrograph of the lumen of a mouse uterus in the first stage of closure. The microvilli from opposing surfaces are interlocking. (Picture by Dr Rosemary Pollard.)

closure' (Figure 3.6) (Nilsson, 1966; R.M. Pollard and Finn, 1972).

Guinea pigs do not appear to undergo this second stage (Green, 1979), and no such stage has been reported in ruminants. The cause of the pronounced changes in the uterine luminal surface is probably the alterations to the cytoskeleton. It is suggested (Luxford and Murphy, 1989, 1992) that the ovarian hormones control the microfilaments underlying the apical plasma membrane (section 3.4.5 on attachment of blastocyst).

Rabbits undergo a very complicated process of preparation for implantation. Division of the epithelial cells occurs several days after ovulation (the rabbit is an induced ovulator

and does not have a cycle) following formation of the corpus luteum. Then, just before the expected time of blastocyst attachment, the luminal epithelial cells join together to form large multinucleated cells (syncytium) (Davies and Hoffman, 1973; Winterhager *et al.*, 1984). The fusion seems to be preceded by the formation of many gap junctions between the lateral surfaces of adjacent cells (Winterhager and Kuhnel, 1982).

(b) Glandular epithelium

Uterine glands are developed as downgrowths of the luminal epithelium. They are coiled structures and are, therefore, usually

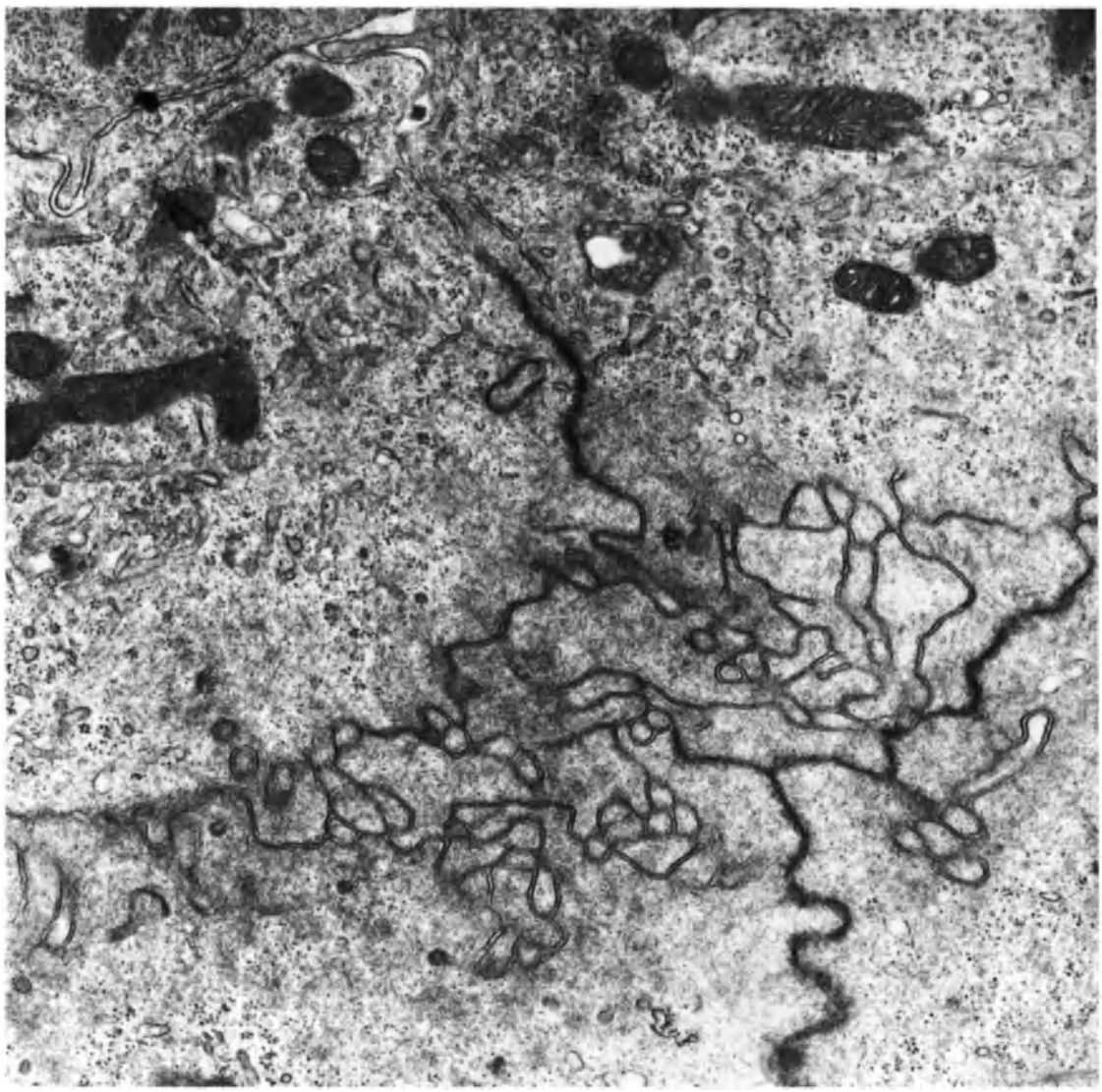

Figure 3.6 Electron micrograph of the lumen of a mouse uterus in the second stage of closure. The microvilli are no longer clearly defined and a very complex channel is formed between opposing surfaces. (Picture by Dr Rosemary Pollard.)

seen in histological sections as round follicles. After the peak of cell division on day 3 of the extended cycle (in the mouse) the cells differentiate to produce a periodic acid–Schiff (PAS)-positive secretion (Finn and Martin, 1971). This secretion is present in the glands in the later part of the luteal phase of the menstrual cycle in women and on day 6 of the extended cycle of mice, which would be after the blastocyst had attached but before chorioallantoic placentation had developed. It is interesting that the gland cells, although developed from the luminal cells, behave so differently in timing of mitosis and type of differentiation.

The secretions of these glands are probably important in animals in which there is a long period between arrival of the blastocyst in the uterus and permanent attachment. The secretion presumably contributes to the histiotrophe (Hoyes, 1972). The glands may also be important in the secretion of some of the specific polypeptides found at certain times in the uterus. An interesting recent finding is that leukaemia inhibitory factor (LIF) is found in the glands on day 4 of pregnancy in mice (Bhatt *et al.*, 1991). Mutant mice unable to synthesize LIF do not implant blastocysts (C.L. Stewart *et al.*, 1992), suggesting an important role in pregnancy. It had been suggested earlier (Aitken, 1976, 1977a) that gland secretions may be involved in activating the blastocyst during delayed implantation. Given and Enders (1980, 1981) have made an ultrastructural study of the glands of the mouse during the peri-implantation period, and have concluded that the mode of secretion is similar to that of other exocrine glands. The fine structure of the glands at various stages of the menstrual cycle has also been studied in women (Gompel, 1964).

(c) Stroma

The main stromal cells are the undifferentiated mesenchymal cells or fibroblasts. In rodents during the luteal phase of the mated cycle (and probably in man), these cells undergo division and are considerably influenced by the ovarian hormones. Their appearance after the burst of division on days 4 and 5 of the mated cycle in the rat has been examined with the electron microscope (Jollie and Benscosme, 1965). Endoplasmic reticulum is abundant and there are observable changes in the nucleoli. In view of the presence of large quantities of collagen, they are presumably actively secreting this substance (Figure 3.7).

However, in most animals, they do not differentiate further except during pregnancy and then only in areas where a blastocyst is present. There they differentiate into decidual cells. In man and a few other species decidualization takes place during the unmated cycle (section 3.5.8 on menstruation).

The stroma also contains blood vessels which undergo changes during the cycle. These are particularly prominent in the primate endometrium, where there is considerable development of the blood vessels. During the luteal part of the cycle the vessels grow towards the lumen and there is an increase in coiling (Daron, 1936; Phelps, 1946; Kaiser, 1948) and blood flow (Prill and Gotz, 1961).

The flow of blood through the uterus of other animals also varies during the cycle. In all animals studied the flow increases at prooestrus and oestrus. In some animals there is a second increase of blood just before the expected time of implantation. This has been shown in several rodents (guinea pig, Markee, 1929; Bacsich and Wyburn, 1940; Garris, 1984; rat, M.F. Williams, 1948; Einer-Jensen, 1977; Garris and Whitehead, 1981; and mouse, Bindon, 1969; Edwards and Milligan, 1987), although not in the ewe (Greiss and Anderson, 1969), rabbit (Krichesky, 1943) or sow (Ford and Christenson, 1979).

An interesting vascular change reported by Lunam and Rogers (1981) is an increase in the number of pericytes around the blood vessels

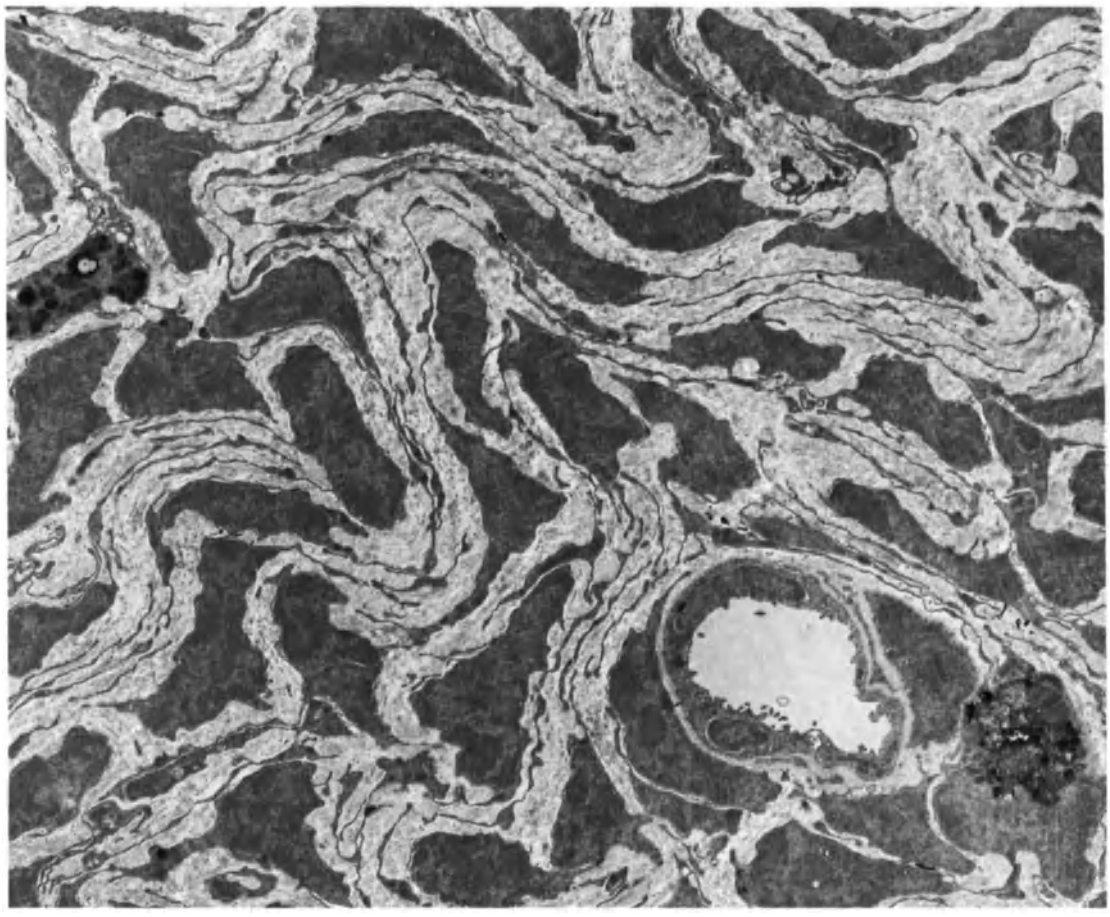

Figure 3.7 Low-power electron micrograph of an area of endometrial stroma of a mouse. Typical fibroblasts are shown.

of the endometrial stroma. These are flattened cells which have been reported in various situations and can be distinguished from the other connective tissue fibrocytes, although their origin or function is not known. It is, nevertheless, very interesting that they appear to increase markedly just before the expected time of implantation.

3.3.6 HORMONAL CONTROL OF DIFFERENTIATION

The same sequence of hormone secretion already shown to control cell division is responsible for directing the differentiation of the tissues. Hitschmann and Adler (1908) were probably the first workers to show that the cyclic changes in the endometrium were related to changes in the ovary. Since then many workers have studied the influence of oestrogen and progesterone on the morphology of the uterus, both with the light microscope (W.M. Allen, 1937; Loeb *et al.*, 1938; Burgos and Wislocki, 1958) and with the electron microscope (Nilsson, 1958; R.M. Pollard and Finn, 1972; Ljungkvist, 1971a–c). There is some variation between species and it is somewhat difficult to bring together all

the data into a coherent picture. The response to one of the ovarian hormones depends very much on the dose given, the period over which it is administered and the relationship to previous or concurrent injections of the other hormone (and possibly non-ovarian hormones). This was shown to be true of the effect of the hormones on mitosis, but is equally true of differentiation.

(a) Luminal epithelium

The uterus of any animal becomes atrophic after ovariectomy (Langston and Robinson, 1935), the epithelium becomes thinner and has an eosinophilic surface. The cytoplasm stains lightly and there are some fat droplets (triacylglycerols, Boshier *et al.*, 1981) in the base of the cells (Alden, 1947). The nuclei are small without prominent nucleoli. After a single dose of oestradiol (20–100 ng) the epithelium becomes thicker and, if appropriate staining methods are used, the luminal surface can be shown to contain alkaline phosphatase (Atkinson and Elftman, 1947; Atkinson and Engle, 1947; Aldeen, 1970). With higher doses, to give a level probably equivalent to pro-oestrus, the lumen fills with fluid (Astwood, 1938), which may escape during fixation and processing so that in cross-section, the lumen is very irregularly shaped and the cells bunched up. The amount of fluid in the uterus varies considerably between species and is particularly prominent in the mouse and rat. Associated with the passage of fluid is the appearance of gaps in the normally complete endothelial lining of capillaries and venues (Ham *et al.*, 1970). The lipid droplets in the base of the cells disappear after oestrogen treatment. Progesterone causes the epithelium to become thicker, with an extensive accumulation of subnuclear fat. After three or more daily injections of progesterone, the lumen closes with interdigitation of microvilli as seen on day 4 of the mated cycle in mice (Martin *et al.*, 1970). Using the electron microscope, it is found that to obtain the second stage of closure as seen on day 5 of the extended cycle it is necessary to administer a small quantity of oestrogen in addition to progesterone; progesterone alone can only induce the first stage of closure in mice. The second stage of closure can, however, only be maintained for a short period, after which the lumen reopens and an electron-dense substance appears in the uterus. At this time the uterine glands are actively secreting, and possibly it is their secretion which causes the morphological changes seen. Pinocytosis in the uterine epithelium is also under hormonal control and requires the same hormonal parameters as are required for implantation (Leroy *et al.*, 1976), as does the formation of pinopodes on the surface of the luminal epithelium (Martel *et al.*, 1991).

(b) Glandular epithelium

Although in the rabbit progesterone stimulates proliferation of the glands, its effect in most animals is to convert the glands into a secretory condition. This has been shown in women (Good and Moyer, 1968) and many other animals *in vivo* and in tissue culture (man, Kohorn and Tchao, 1969; guinea pig, Everett, 1962, 1963).

In the mouse daily injections of progesterone alone stimulate only a small amount of secretion by the glands even after 9 days' treatment. However, if a small quantity of oestradiol (10 ng) is added to the daily injections of progesterone, then the glands secrete, producing maximum secretion on the fifth day (Finn and Martin, 1976).

(c) Stroma

In women, stromal cells differentiate into decidual cells under the influence of progesterone or synthetic progestins (Ober *et al.*, 1964). However, in most animals differentiative changes in the stroma are more limited in response to hormones. The cells increase in

size somewhat and the nuclei (and nucleoli) get larger (Hooker, 1945; Tachi *et al.*, 1974). Under the influence of progesterone there is also evidence of increased activity in the cytoplasm (Greenwald and Everett, 1959; Brinsfield and Hawk, 1974) with an increase in rough endoplasmic reticulum and free ribosomes. Other changes in the organization and activity of the uterine stromal cells have been described (Wischik and Rogers, 1982; Rogers and Wischik, 1983). Blood vessels are also affected by the ovarian hormones so that blood flow is increased in preparation for implantation (Bacsich and Wyburn, 1941; Gilman, 1941; Salvatore, 1968; W.M. Dickson *et al.*, 1969; Ford *et al.*, 1977; Edwards and Milligan, 1987).

Clearly, the hormones secreted before implantation affect the stroma, however it is probably not correct to call these 'differentiated' changes, such as the decidual changes to be described later.

3.3.7 CELL DEATH

Cell death in a tissue is very difficult to measure quantitatively. Cells that are dying can be identified in histological sections from the shape and staining properties of the nuclei. However, dead cells are soon broken down and removed, probably at varying speeds, and therefore it is not possible to get a reasonably quantitative assessment of the number of cells dying over a period of time by simply counting the dead or dying cells. From histological studies in mice it is apparent that there is a significant amount of cell death in the endometrium during the mated cycle (Martin *et al.*, 1973). This is necessary to remodel the uterine lumen between oestrus and implantation. It is also obvious that the big increase in cell numbers in the stroma following the onset of progesterone secretion must be followed, if implantation does not take place, by a reduction in cell numbers before the start of the next cycle.

Several possible approaches to the assessment of the extent of cell death are possible. Each cell contains a characteristic quantity of DNA in the nucleus so that any change in total DNA in a tissue will represent a change in cell numbers. If cells are not proliferating, then a drop in DNA would represent cell removal and thus give some estimate of cell death. There is, however, the problem of the migration of cells from the blood. Under most conditions, this is not likely to be very great but it might complicate the picture. The problem with regard to this approach to the uterus is the difficulty of separating the various cells and tissues for biochemical analysis, although some success has been achieved at least in separating the endometrium from the myometrium and the luminal epithelium from the stroma (Fagg *et al.*, 1979; Glasser and Julian, 1986).

An alternative approach to the problem (Finn and Publicover, 1981) is to utilize the findings discussed earlier, that the different tissues of the endometrium undergo mitosis at different times and in response to different hormone treatments. Thus, the cells of each tissue will be synthesizing DNA and taking up thymidine at different times. When an injection of tritiated thymidine is given to an animal, cells in the S phase of mitosis take up the nucleotide and become radioactively labelled. This can be confirmed by making autoradiographs of the sections of the uterus after such an injection when the labelled cells show up clearly (Finn and Bredl, 1977). Thus, if an injection of tritiated thymidine is given to ovariectomized mice after two injections of 100 ng of oestradiol, then most of the luminal epithelial cells will be labelled and none of the stromal, whereas if the thymidine is given following treatment with oestrogen and progesterone to mimic the preparation for implantation then the label is found predominantly in the stromal cells.

With this technique, it is possible to identify a distinct population of cells and to follow their fate. It is assumed that any fall in the level of radioactivity in the uterus indicates

that cells from the tissue which was labelled have died and been removed. To estimate the number of labelled cells, it is easy to measure total radioactivity in the uterus by scintillation counting. As expected, it was found (Finn and Publicover, 1981) that the uteri of animals given tritiated thymidine after two daily injections of oestradiol had a very high level of radioactivity when killed soon after, but this had dropped to about 25% by the third day after injection, indicating that many of the luminal epithelial cells had been removed. If the oestrogen treatment was maintained, however, the level of radioactivity did not decrease, demonstrating that it is the decline in oestradiol levels which causes the cells to die.

When the thymidine is injected so as to label the stromal cells, i.e. after progesterone treatment, then the radioactivity is maintained for 2 days and then declines over the following 5 or 6 days. The reduction is less if the progesterone treatment is continued, but is still very significant. If, however, the uterus is stimulated to induce the decidual cell reaction (section 3.5) then the level of radioactivity is maintained. It was concluded that many of the stromal cells which are formed following mitosis in preparation for implantation are in an unstable condition in which they can either decidualize or die. Maintenance of a large population of undecidualized stromal cells does not occur.

In women decidualization occurs during the menstrual cycle, but if pregnancy does not follow there is overt breakdown of endometrial tissues causing menstruation (section 3.5.8). Whilst the extent of cell death has not been assessed quantitatively during menstruation it must be very high.

Clearly, there is no really satisfactory way of accurately measuring cell death at the moment. Nevertheless, it is clear that it is an important factor involved in the preparation of the uterus for implantation.

3.4 IMPLANTATION OF THE BLASTOCYST

3.4.1 LOCATION OF THE BLASTOCYST

Whether the blastocyst remains small and ultimately comes to lie in the wall of the uterus, or increases in size and fills the uterine lumen, the first stage of implantation is apposition of the trophoblast against the luminal surface of the uterine epithelium. Before this happens however, the blastocyst has to be positioned both with regard to its place along the length of the uterus, the orientation of the embryonic disc, and, in the case of the blastocysts which remain small, with regard to its position across the axis of the uterus. As discussed in the previous section, the uterus, even in the absence of a blastocyst, closes down during the mated cycle, and in the case of the mouse and rat the lumen appears as a slit orientated in a mesometrial–antimesometrial direction (Figure 3.4).

When blastocysts are present in mice and rats they locate themselves fairly evenly along the length of the uterus at the antimesometrial side of the lumen with the embryonic disc facing towards the mesometrium. In other species the location is different but characteristic for the species. This spacing and locating mechanism is also found in the uteri of animals undergoing delay of implantation (McLaren, 1968).

Three interesting questions present themselves:

(1) What forces operate on the blastocysts to space them along the length of the uterus?
(2) What causes the blastocyst to position on the antimesometrial side of the lumen?
(3) What is the mechanism causing the embryonic knob to face in a particular direction with regard to the mesometrium?

All of these problems have been investigated but no conclusive answers have been reached. (For a more detailed discussion of

the possible mechanisms see Mossman, 1971; Wimsatt, 1975)

When a small quantity of oil is injected into the uterine lumen to stimulate an implantation reaction (Finn and Keen, 1963), it breaks up into small droplets and a decidual response is always formed round the antimesometrial side of the lumen (Finn and Hinchliffe, 1964). The oil droplets are, however, found randomly along the uterine slit (Finn and Pope, 1989). However, pieces of muscle or tumour cells placed in the uterus become located antimesometrially (Wilson, 1960, 1963). There seem to be two factors operating: a locating mechanism and a preferential sensitivity for stimulation of the decidual cell reaction. Martin (1979) has studied the locating mechanisms in the mouse and has suggested that the circular muscle layers of the myometrium are active at the time of implantation under the influence of progesterone, and it is their contraction which forces the blastocyst towards the antimesometrial side. Martin points out that 'in transverse sections the antimesometrial extremity of the lumen is usually closer to the centre of a circle formed by the circular muscles than the mesometrial extremity. A body lying mesometrially would therefore receive asymmetric forces from the circular muscle and would tend to move to the centre of symmetry of the antimesometrial cleft.' An alternative hypothesis (Finn, 1989) suggests that the surface of the luminal epithelium on the antimesometrial side is preferentially organized both for reception of the decidual stimulus and for the attachment of the blastocyst. Myometrial activity would cause random movement of the blastocyst, which when it came in contact with the antimesometrial epithelium would not only stimulate a decidual reaction but become attached there. Oil droplets, whilst stimulating a decidual reaction, would not become attached and therefore are still free to move.

Spacing of blastocysts along the length of the uterus ensures that each blastocyst has sufficient space in which to develop. When there are many blastocysts this means more or less even spacing, although this is not necessarily so when there are only a few. Several suggestions have been proposed to account for the spacing. Mossman (1937) proposed that as each blastocyst starts to implant it sets up around itself an inhibitory zone which prevents other blastocysts implanting nearby. This hypothesis was challenged by Boving (1954) and McLaren and Michie (1959), who suggested that on arrival in the uterus the blastocysts are subjected to pressures exerted by muscular activity, which have the effect of distributing them along the length of the uterus. Evidence in favour of this view comes from the work of Pusey *et al.* (1980), who showed that relaxin, a hormone known to affect the activity of the myometrium, if given before implantation, upsets the normal distribution of blastocysts along the length of the uterus.

Whilst it seems very likely that muscular movement plays some role in the early distribution of the blastocysts, it may not by itself be sufficient to obtain the degree of even spacing attained when there are, for instance, 17 or more ova in the uterus of a mouse. A further mechanism has therefore been proposed in which, after an initial approximate distribution by muscular activity, more accurate spacing is achieved by growth of the uterus between implantation sites (Hammond, 1935; Reynolds, 1949; Finn, 1968). Certainly a very rapid increase in length of the uterus at the time of implantation has been noted, which might be due to increased cell proliferation (Finn, 1968) or to tissue hydration following a local increase in endometrial microvascular permeability at the implantation sites (Rogers *et al.* 1982a). However, Hamilton (1973) contests the view that there is growth of the uterus at the time of implantation.

The constant orientation of the embryonic knob with regard to the mesometrium and thus the entry point of the blood vessels is

associated with the final form of the placenta (Mossman, 1937). This is probably not so important in animals with placentae which involve the whole uterine surface (such as the pig and horse) or specialized areas over the whole surface (such as the cotyledenous placenta of the sheep, cow and goat). Consideration of the possible forces operating to fix the position of the embryonic disc led Kirby *et al.* (1967) to propose that once the blastocyst had been fixed in position the embryonic disc moved round the trophoblast shell to obtain its correct position. Gardner (1975) tested this hypothesis by growing blastocysts in culture medium and labelling the cells abutting the embryo with particles of melanin. He then transferred them to recipients (prepared hormonally to receive them). Once inserted, they orientated themselves correctly but the melanin-labelled cells were still in contact with the embryonic knob. It seems very unlikely therefore that the latter had migrated round the trophoblast and suggests that whatever causes the orientation acts on the whole blastocyst.

3.4.2 ACTIVATION OF THE BLASTOCYST

The arrival of the blastocyst in the lumen of the uterus is a crucial stage of pregnancy. For further growth of the embryo it is necessary for the blastocyst to become fixed against the wall of the uterus so as to tap the resources in the maternal bloodstream more directly. In most animals this happens at a predetermined time, soon after arrival of the blastocyst in the uterus, and if it does not the embryo will die. A few animals, however, can maintain the blastocysts in a condition of delayed implantation and embryonic diapause for extended periods (Lataste, 1891). During this time protein synthesis and metabolism of the blastocyst are reduced (Weitlauf, 1973, 1985). This latter can be shown by the very low level of carbon dioxide production (Menke and McLaren, 1970), the cessation of cell division (McLaren, 1968) and the reduction in RNA synthesis (Gulyas and Daniel, 1969). Some stimulus is necessary to bring the blastocyst out of this dormant condition. The ability of the blastocysts of some animals to stay in this state of suspended animation is very interesting. However, it is probably not a general property of blastocysts, and it is unlikely that activation is in general necessary for embryonic development. Findings about activation of rodent blastocysts should not therefore be extrapolated too freely to other species.

The mechanisms whereby the blastocyst is activated have been studied mainly in small rodents in which the condition of embryonic diapause occurs during lactation or can be easily induced artificially by removing the ovaries early in pregnancy and maintaining the animals on progesterone (Cochrane and Meyer, 1957). Implantation and activation of the blastocyst can be induced in such animals by the injection of small quantities of oestradiol – the same regimen required for the induction of the second stage of closure. Although there can be no doubt that this causes changes in the endometrium which lead to attachment of the blastocyst, it has been suggested that oestrogen also acts directly on the blastocyst to cause activation (D.M. Smith, 1968). This work has been criticized (Wu and Meyer, 1970; Weitlauf, 1973), and it is now generally thought that the oestrogen acts first on the endometrium, which then activates the blastocyst. There is less agreement concerning whether activation involves the turning off of an inhibitory influence of the uterus or the production of a stimulatory factor. It is also possible that the change in the surface of the luminal epithelium which allows attachment of the trophoblast is sufficient to activate the blastocyst.

If an unimplanted blastocyst is transferred from the uterus to another organ, for example the eye (Fawcett *et al.*, 1947), kidney (Fawcett, 1950; Kirby, 1960), testis (Kirby, 1963a; Mayer and Duluc, 1966) or spleen

(Kirby, 1963b), it continues its development, indicating that it is not reliant on a specific uterine factor. Similarly, if blastocysts from animals in delay are grown in tissue culture medium containing serum and amino acids they resume development (Gwatkin, 1966; Psychoyos and Bitton-Casimiri, 1969; Bitton-Casimiri *et al.*, 1976). It is clear from these and other findings that the uterus in delay either produces something inhibitory to development of the blastocyst or fails to provide it with necessary nutrients.

As the embryos of the majority of species develop continuously from fertilization to gestation, it seems likely that inhibition of blastocyst development, in those few species in which embryonic diapause is necessary for reproductive success, would have been superimposed during evolution as an active mechanism. Inhibitory substances have been demonstrated in washings from the uteri of animals in delay (Psychoyos and Bitton-Casimiri, 1969; Weitlauf, 1973; Aitken, 1977a,b; Weitlauf, 1978). Furthermore a finding which indirectly suggests that the uterus produces an inhibitory substance is that delayed blastocysts can be induced to implant by the injection into the mother of the drug actinomycin D (Finn, 1974; Camus *et al.*, 1979). It is suggested that this drug inhibits the synthesis of a newly synthesized protein of approximate molecular weight 7000 daltons (J.W. Pollard *et al.*, 1976), which is responsible for the dormant condition of the blastocyst. Recently, from a study of endometrial protein changes during sensitization for implantation, Mulholland and Leroy (1989) have also concluded that receptivity is effected by repression of certain proteins.

On the other hand, work on animals showing obligatory delay suggests that activation is brought about by increased nutrients in the uterus (protein, ferret, J.C. Daniel, 1970; hexoses, roe deer, Aitken, 1974). Probably the mechanisms for maintaining the blastocyst in diapause differ between those species in which it has evolved as a facultative response to lactation and those in which it is an obligatory part of the reproductive process.

Whether specific uterine secretions are necessary for normal blastocyst development in those species which do not undergo delay of implantation is undecided. Provided the correct nutrients are available, ova grow and develop normally in culture medium (McLaren and Biggers, 1958). However, a specific uterine protein called either 'blastokinin' (Krishnan and Daniel, 1967) or 'uteroglobin' (Beier, 1968) has been isolated in the rabbit, and it has been suggested that it regulates blastocyst development. No such regulator has been shown in other species (e.g. sheep, Staples *et al.*, 1976).

3.4.3 HORMONAL CONTROL OF IMPLANTATION

The necessity of the ovarian hormones for implantation has been known for a very long time (Corner, 1921). Ablation of the corpus luteum was shown to cause failure of implantation (Corner, 1928), which could be reversed by the administration of extracts of the corpus luteum (W.M. Allen and Corner, 1929), showing clearly the necessity for progesterone. This has been confirmed by the demonstration that antibodies to progesterone will block pregnancy (Rider *et al.*, 1985, 1986). Krehbiel's (1941) discovery that oestrogen could precipitate implantation of mouse blastocysts held in delay during lactation led to work indicating a role for oestrogen in implantation, at least in the mouse.

Two groups of workers showed that, whilst removal of the ovaries always prevented implantation in rats, the ability of exogenous progesterone alone to allow implantation when administered after ovariectomy depended on the time at which the ovariectomy was performed (Canivenc *et al.*, 1956; Cochrane and Meyer, 1957). If the ovaries were removed from mated rats or mice before a critical time then a small

amount of oestrogen had to be administered in addition to progesterone. However, the importance of this so-called nidatory oestrogen does not seem to be universal – many species require only progesterone during the luteal phase for implantation (ewe, Bindon, 1971; guinea pig, Deanesly, 1960; hamster, Orsini and Meyer, 1959; Harper *et al.*, 1966, rabbit, Kwun and Emmens, 1974; ferret, Wu and Chang, 1973; Mead and McRae, 1982; weasel, Shelden, 1973; skunk, Mead *et al.*, 1981). The situation in primates is equivocal. Some recent work suggests that there may be a need for luteal phase oestrogen in monkeys (Ravindranath and Moudgal, 1989).

It is also likely that the oestrogen secreted during pro-oestrus is important in preparing the uterus for implantation. Pro-oestrous oestrogen plays a role in controlling cell proliferation and differentiation in the endometrium and affects the timing of sensitivity to implantation in the mouse (Finn and Pollard, 1973). Possibly pro-oestrous oestrogen plays a more crucial role in species which implant following a set time interval after ovulation (Marcus and Shelesnyak, 1967; Finn and Martin, 1970; Moore and Miller, 1976), whereas in rats and mice, in which there is a variable timing of implantation associated with the presence of lactating pups, nidatory oestrogen has become important. (For a detailed review of the hormonal control of implantation see Psychoyos, 1974.)

The state of blastocyst acceptance or sensitivity is maintained for only a few hours (the so-called implantation window). At other times the uterus is hostile to the blastocyst. Such a condition of insensitivity was predictable from the early work on sensitivity to a decidual stimulus (Loeb, 1908a; W.M. Allen, 1931).

The critical timing of uterine sensitivity to the implantation of blastocysts was shown originally in mice by McLaren and Michie (1956), who transferred fertilized ova to pseudopregnant mice on succeeding days of pseudopregnancy and found that attachment of blastocysts would only occur over a very limited period. This was subsequently confirmed in rats (Noyes and Dickman, 1960).

During the oestrous cycles of animals which have a spontaneous luteal phase, the demise of the corpus luteum occurs very soon after the time when full sensitivity of the uterus had been attained and the fall in progesterone would be sufficient to render the uterus insensitive to the blastocyst. However, in animals with an induced luteal phase (pseudopregnancy) the progesterone levels remain high until well after the expected time of implantation, but the uterus nevertheless still becomes insensitive.

Most of the experimental work on the attainment of sensitivity and insensitivity has been carried out on species in which insensitivity occurs in spite of high progesterone levels, and it may not be valid to extrapolate results from these animals to species in which such a condition would not naturally exist (for example man).

Experimental work on ovariectomized mice has shown that if they are treated with hormones on a schedule of injections, which causes morphological changes in the endometrium similar to those found in pregnancy or the extended oestrous cycle, then the uterus will respond to a blastocyst or artificial decidual stimulus 4–6 Hafter the nidatory oestrogen injection but will then become refractory to such a stimulus even though progesterone treatment is continued (Finn, 1966a; Psychoyos, 1963; Finn and Martin, 1969a, 1972a; Finn *et al.*, 1991). Associated with the refractory state is the presence in the lumen of an electron-opaque substance which may be the result of secretion of the glands. The luminal surface of the epithelial cells loses its typical appearance of the second stage of closure. Whether such a refractory period with its associated morphological changes occurs in animals with a spontaneous luteal phase if progesterone is maintained artificially beyond the time of sensitivity is not known.

A similar condition of insensitivity to an implantation stimulus is produced if high doses of oestrogen are given during the luteal phase. It has been known for a long time that pregnancy can be interrupted by the administration of oestrogens before the time of implantation (Parkes *et al.*, 1938). Unlike progesterone, excess levels of which, provided the dosage is above the threshold, have no effect on uterine sensitivity, the response to nidatory oestrogen is critically dependent on the dose given (Finn and Martin, 1969b). Morphological examination of animals given high doses of nidatory oestrogen suggests that the onset of the closure reaction of the luminal surface is accelerated, which may be a factor causing insensitivity (R.M. Pollard and Finn, 1972).

3.4.4 CYTOKINES AND IMPLANTATION

Implantation involves a series of changes in the uterus and blastocyst involving endocrine, autocrine and paracrine control mechanisms. The role of growth factors and oncogenes in cell proliferation has been discussed earlier and the role of cytokines in the immune response will be discussed later. Although there may be redundancy in the system it is likely that removal of any of these would affect the course of implantation.

Some of these growth factors and other cytokines must also be involved in differentiation of the endometrium and attachment of the blastocyst. Two in particular have been implicated as playing a crucial role in implantation – leukaemia inhibitory factor (LIF) (C.L. Stewart *et al.*, 1992) and colony-stimulating factor 1 (CSF1) (J.W. Pollard *et al.*, 1991). Mice containing mutant genes rendering them unable to synthesize the cytokine have been developed, and in both cases pregnancy is compromised. It is suggested that CSF1 is necessary for placental development (Pollard *et al.*, 1987; Pampfer *et al.*, 1991, 1992), although the stage of placental development affected is not clear. The suggestion seems to be that endometrially produced CSF1 is essential for the growth of trophoblast cells. It is difficult to reconcile this with the fact that the trophoblast grows in animals in which transcription has been stopped by the administration of actinomycin D (Finn and Bredl, 1975).

The finding that blastocysts grow actively in other tissues (discussed earlier), although not ruling out the necessity for the presence of CSF1, suggests that it is not having a controlling influence. It would be interesting to know whether such ectopic blastocysts would grow in mutant mice devoid of CSF1. Further work is also needed to find the stage of implantation at which failure occurs. Actinomycin D, which presumably blocks the transcription of genes for CSF1, does not block attachment of the blastocyst or the pontamine blue reaction but does block apoptosis of the luminal epithelium and decidualization. Clearly, more biological information is needed before one can be sure that the uterus is producing cytokines which control the growth of the blastocyst.

Both LIF and CSF1 are cytokines originally discovered for their effects on the haemopoietic and immune systems. Colony-stimulating factor controls the recruitment of macrophages and other leucocytes to the uterus and is itself under the control of oestrogen and progesterone (G.W. Wood *et al.*, 1992). The role of bone marrow-derived cells in implantation is discussed in the section on the decidua, but whether there is any relationship between their presence and successful pregnancy is at present unknown.

3.4.5 ATTACHMENT OF THE BLASTOCYST TO THE UTERUS

On arrival in the uterus the blastocyst is normally still encased in the zona pellucida. In most animals this is removed before there is any contact between the trophoblast and endometrium. However, in the guinea pig, tongues of trophoblast penetrate through the

zona to make contact with the luminal epithelium whilst the zona is largely intact (Blandau, 1949). The rabbit also differs from the majority in secreting around the blastocyst additional coats of glycoprotein-like materials, which subsequently have to be removed before attachment, probably by proteases in the uterine secretions (Kirchner, 1975).

Removal of the zona appears to be largely brought about by enzymes in the uterine luminal fluid (McLaren, 1969, 1970; Rosenfeld and Joshi, 1981). There is some suggestion that the blastocyst contributes to the activation of this enzyme, and indeed blastocysts can hatch from the zona even in the absence of the proteolytic enzymes. This happens in delayed implantation (McLaren, 1968), when empty zonas can sometimes be seen in the uterus from which blastocysts have hatched without prior dissolution of the zona (Alloiteau and Psychoyos, 1966; McLaren, 1970). Similarly, if blastocysts are delayed in the oviducts, they will hatch, although at a later time than normal (Orsini and McLaren, 1967).

After emerging from the zona the blastocyst continues to have a surface coat of glycoprotein. This may be very thick, as found on the blastocysts of the rabbit and ferret, or only visible after the use of special stains, such as concanavalin A or ruthenium red (rodents; Enders and Schlafke, 1974; Nilsson, 1974; Wu and Chang, 1978).

The initial contact between the trophoblast and the luminal surface of the uterine epithelium has been studied in several animals (human, Aplin, 1991; rats, Nilsson, 1966; Enders and Schlafke, 1967, 1969; Schlafke and Enders, 1975; and mice, Mayer *et al.*, 1967; Reinius, 1967). Attachment involves changes in the chemical and physical nature of the contacting membranes of both the uterine epithelial cells (Murphy *et al.*, 1982b) and the trophoblast (although it only seems to be the mural trophoblast). The morphological change in the lumen of the mouse uterus which occurs at the expected time of implantation brings about closure of the uterine lumen and requires the intervention of a small quantity of oestrogen against a background of high levels of progesterone. It does not take place during delayed implantation in the mouse and is not dependent on the presence of a blastocyst, although there is some evidence that contact of the trophoblast may accelerate onset of the change (R.M. Pollard and Finn, 1974). When a blastocyst is present a similar condition of very close contact is formed between the trophoblast and uterine luminal surface (Figure 3.8) (Nilsson, 1967; Hedlund *et al.*, 1972; Ljungkvist, 1972).

Because it is not possible to wash the blastocysts out at this stage, it is called the adhesion stage.

The chemical and physical basis of this adhesion between the blastocyst and uterus is still being actively investigated (Anderson, 1989). It is assumed that there must be some sort of adhesive force holding the cell membranes together. Cells are held apart by the negative charge on their surfaces, and reduction of this is thought to be a necessary step in attachment. Attempts have been made to assess changes in negativity, both at the surface of the luminal epithelium and the blastocyst, using electron-dense probes, for example ruthenium red, alcian blue, colloidal iron hydroxide and polycationic ferritin (Holmes and Dickson, 1973; Nilsson *et al.*, 1974; Jenkinson and Searle, 1977; Hewitt *et al.*, 1979; Guillomot *et al.*, 1982; Anderson and Hoffman, 1984). In some animals a change has been demonstrated, but not in others.

The cause of the change in charge on the cell membranes is probably associated with the complex carbohydrate moieties of the glycoprotein, glycolipids and glycosoaminoglycans located at the cell surface in the glycocalyx (Wordinger and Amsler, 1980; C.R. Murphy and Rogers, 1981; Chavez and Anderson, 1985; Whyte and Allen, 1985; Chavez, 1986; Aplin, 1991). It has been suggested that the reduced negativity may be

caused by decreased amount of sialic acid residues (Nilsson *et al.*, 1974; Jenkinson and Searle, 1977).

Some authors have reported a thinning of surface coat material before attachment (Salizar-Rubio *et al.*, 1980), while others have been unable to show such an effect (Enders *et al.*, 1980; Guillomot *et al.*, 1982). Changes in the chemical nature of the surface carbohydrates have been studied by assessing the binding of various lectins and antibodies (human, M.C. Lee and Damjanov, 1985; Duncan

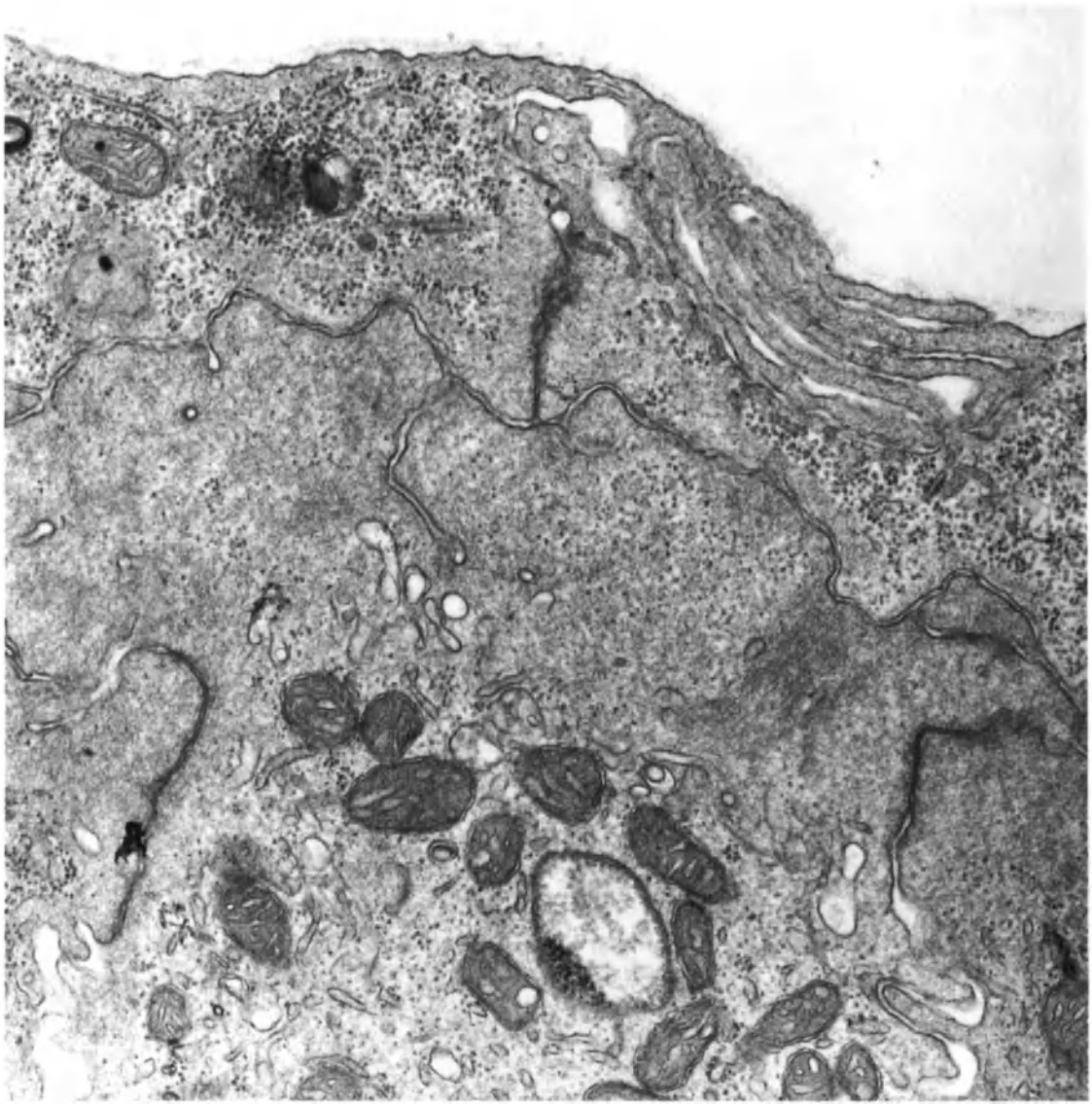

Figure 3.8 Electron micrograph of the junction of a trophoblast cell (top) with the uterine epithelium at the adhesion stage of implantation in the mouse. The interlocking microvilli have flattened out and very close contact has been established between the surfaces of the apposing cells. (Picture by Dr Rosemary Pollard.)

et al., 1988; cow, Wordinger and Amsler, 1980; rat, C.R. Murphy and Rogers, 1981; C.R. Murphy and Turner, 1991; and mouse, Wu and Chang, 1978; M.C. Lee *et al.*, 1983). Chavez (1986) tested the binding of several lectins to both surfaces and showed changes at the time of adhesion in only one lectin, succinylated wheat germ agglutinin. From these studies he suggests that at the time of attachment there is a change in *N*-acetylglucosamine on the cell surfaces.

Changes in cell-surface molecules in response to ovarian hormones have also been demonstrated. The cell adhesion molecule (CAM 105) is controlled by oestrogen and progesterone (Svalander *et al.*, 1987, 1990) in rats as are lacto-*N*-fucopentose (Kimber and Lindenberg, 1990) and lactosaminoglycans (Barbiarz and Hathaway, 1988) in mice. Recently, Potter and Morris (1992) have demonstrated changes in the distribution of heparin sulphate proteoglycan in response to oestradiol (they were unable to show any response to progesterone, but as they used an extremely low dose, 1.25 μg per mouse, this is not surprising).

Attempts have been made to isolate and purify the plasma membranes from the luminal epithelial cells and investigate their protein content (rabbit, Lampelo *et al.*, 1985; pig, Mullins *et al.*, 1980). This has shown that at the time of acquisition of receptivity to a blastocyst there is a change in protein composition of the endometrial plasma membranes. It is suggested (Lampelo *et al.*, 1985) that this may involve a shift in the number, distribution or configuration of some of the molecules on the surface. Similarly, Anderson *et al.* (1986) have shown that three new polypeptides can be identified in endometrial epithelial cells from rabbit uteri at the time of sensitivity to implantation. C.R. Murphy and Martin (1985) have suggested a different organization of cholesterol in the apical plasma membrane at the time of implantation.

It is clear that the adhesion between trophoblast and uterine luminal epithelial cell surface (and the second stage of closure) involves complex molecular changes. These changes do not occur in all species, and have indeed been studied in relatively few. It is nevertheless very relevant that the uterine changes in those animals in which they do occur take place during the extended oestrous cycle and produce adhesion between apposing surfaces along the length of the uterus. In pregnancy, it is only in those areas where a trophoblast is in contact with the epithelial surface that decidualization is triggered in the stroma, indicating that the cellular contact between trophoblast and epithelial surface must be different from that between apposing epithelial surfaces. The nature of the triggering stimulus is discussed later in section 3.4.6.

3.4.6 EPITHELIAL RESPONSE TO THE BLASTOCYST

In many species the close attachment of the trophoblast to the luminal epithelium is the final stage in the joining together of the fetal and maternal tissues (e.g. pig and horse). However, in some species there are further developments in the epithelium which bring about a closer fetal–maternal union. The changes in the domestic ungulates are described briefly in section 3.2 on species variation, and details will be found in Chapter 4. In this section the changes associated with implantation in species in which penetration of the trophoblast into the uterine stroma takes place will be described.

Deep implantation of the blastocyst into the wall of the uterus can come about either by degeneration and removal of the uterine epithelium which lies between the stroma and the blastocyst, with the trophoblast taking a passive role, or by the trophoblast actively invading through a healthy epithelium. The former appears to take place in rats and mice (Finn and Lawn, 1968) while the latter occurs in guinea pigs (Green, 1979),

ferrets and probably women (Knoth and Larsen, 1972; Lala and Graham, 1990).

It has been known for a long time that the uterine luminal epithelium, which in the rat and mouse is in contact with the blastocyst, undergoes degeneration, and that the cells of the trophoblast phagocytose the dead cells (Galassi, 1967; Mulnard, 1967; Finn and Lawn, 1968). However, there was dispute as to whether the trophoblast actively brought about the death of the epithelial cells or whether death was due to activity within the epithelial cells which was simply triggered by the attachment of the trophoblast. Such programmed cell death plays an important part in the modelling of tissues during embryogenesis (Saunders, 1966).

Most of the evidence points to the latter. If the drug actinomycin D is given to mice just before implantation the epithelial cells surrounding the blastocyst do not die (Finn and Bredl, 1973, 1975). This suggests that the drug, which stops transcription of DNA in the nucleus, is preventing the release of information which would bring about epithelial cell death. The blastocyst, on the other hand, does not appear to be affected by the drug and both the trophoblast and embryonic knob develop surrounded by intact uterine epithelium. At a later stage the trophoblast may send out processes between the luminal epithelial cells (Finn and Bredl, 1973). Another relevant finding is that when a decidual cell reaction is initiated by the instillation of a droplet of oil in the uterine lumen, the epithelial cells on the antimesometrial side of the lumen, but not those on the mesometrial side, degenerate, again indicating that the breakdown of the cells is spontaneous and only requires the stimulus to trigger it off. The instillation of oil into the uterine lumen of a mouse which has not been prepared hormonally for implantation does not cause the cells to die, indicating that the death of the cells is not due to any trauma caused by the oil but is part of the implantation reaction triggered off by it (Finn and Hinchliffe, 1965).

The rabbit blastocyst demonstrates a very unusual method of transit to the endometrial stroma. At the time of implantation areas of the trophoblast become syncytial and pegs of tissue pass through the blastocyst coverings to make contact with the uterine epithelial cells. Here the apposing surfaces of the two tissues fuse to form a single syncytium. As discussed earlier, the luminal epithelial cells, as part of their hormonal preparation for implantation, form a syncytium, so that there is now a combined maternal–fetal syncytium; with nuclei from both tissues. Soon the fetal nuclei become predominant and the maternal nuclei disappear so that the blastocyst has in effect penetrated the uterine epithelium (Larsen, 1961; Enders and Schlafke, 1971; Steer, 1971).

The other method of trophoblast penetration, which Schlafke and Enders (1975) refer to as intrusive implantation, can be seen in several species, but has been mainly studied in the ferret and guinea pig (von Spee, 1893; Samson and Hill, 1931; Blandau, 1949; Enders and Schlafke, 1972). In this method, processes from the trophoblast penetrate between the uterine epithelial cells with no apparent damage to the cells, beyond the breaking down of the apical junctions between them. The process is very rapid and few studies have been made of blastocysts in the process of passage through the epithelium (Schlafke and Enders, 1975; Green, 1979). Subsequently, the trophoblast, which is largely syncytial, penetrates deeply into the stroma and surrounds blood vessels and glands (Enders and Schlafke, 1971).

It is likely that women and some of the other primates undergo intrusive implantation. Unfortunately, however, the few descriptions of human implantation are of a fairly late stage, and intrusion can only be inferred (Knoth and Larsen, 1972; Hertig and Rock, 1941; 1945). Recent *in vitro* studies also point in this direction (Lindenberg *et al.*, 1986). Similarly the few detailed studies of other primates provide suggestive but not

decisive evidence for intrusive implantation (chimpanzee, Heuser, 1940; rhesus monkey, Reinius *et al.*, 1973; Heuser and Streeter, 1941).

Following the breakdown of the luminal epithelial cells surrounding the blastocyst, the trophoblast cells become very active, first penetrating the epithelial basement membrane (Schlafke *et al.*, 1985; Blankenship and Given, 1992) and then invading and ingesting the stromal cells (Bevilacqua and Abrahamsohn, 1989). Protease enzymes are probably involved (Kubo *et al.*, 1981; Lala and Graham, 1990), especially matrix metalloproteinase (collagenase, gelatinase and stromelysin) (Salamonsen *et al.*, 1991). *In vitro* studies suggest that fibronectin and laminin (Armant *et al.*, 1986; Sutherland *et al.*, 1988; Romagnano and Barbiarz, 1990) or heparin/heparin sulphate (Farach *et al.*, 1987) are involved in the attachment and invasion. The adhesion molecule CAM 105 has also been implicated (Julian *et al.*, 1992; Svalander *et al.*, 1990).

However, there is good evidence that apart from trophoblast invasion the stromal cells, which by now are decidualized, are very active in modelling of the implantation chamber. They appear to have a greater capacity to degrade extracellular matrix *in vitro* than either rat or mouse trophoblast cells (Welsh and Enders, 1989). The fact that implantation chambers form in animals stimulated with oil (Finn and Hinchliffe, 1965) also indicates that much of the apoptosis and remodelling of the stroma is independent of the trophoblast.

3.5 THE DECIDUAL CELL REACTION

In those species in which the blastocyst comes to lie within the stroma, the cells and blood vessels of the stroma undergo a marked transformation into a specialized tissue called the decidua.

3.5.1 HISTORICAL SURVEY

Interest in the decidua as part of the human placenta probably precedes interest in implantation of the ova. More than 200 years ago Hunter (1794) in his treatise on human gestation described the decidua as follows:

> This is a membrane of a very peculiar nature, the knowledge of which throws great light upon the contents of the pregnant uterus, and upon the connection between the mother and child. It is the outer membrane of the uterus.

By this description of the human decidua and the earlier pictures (Figure 3.9) he published of the gravid uterus (Hunter, 1777), Hunter first showed the maternal origin of the decidua and can be said to be the father of over 200 years of research on this fascinating tissue.

Although primitive microscopes were available in the eighteenth century, the study of the histology of tissues did not get under way until the nineteenth century. What Hunter described was a tissue or membrane, clearly visible macroscopically, and it needs to be stressed even today that the decidua is a tissue, consisting of several cell types and specialized blood vessels.

Like much scientific progress, advance in knowledge of the decidua has gone hand in hand with advances in technology. The advent of the compound microscope and techniques for the sectioning of tissues, originally using hand-held razors and subsequently using the microtome, allowed the cellular nature of the tissue to be investigated. The demonstration of the specialized decidual cells and their very large size must have come in the second half of the nineteenth century. In his review of anatomy in 1873, Sir William Turner refers to the 'well known colossal decidual cells'. In fact the cells seem to have been widely studied during the later part of the nineteenth century, and it was during this time that they were

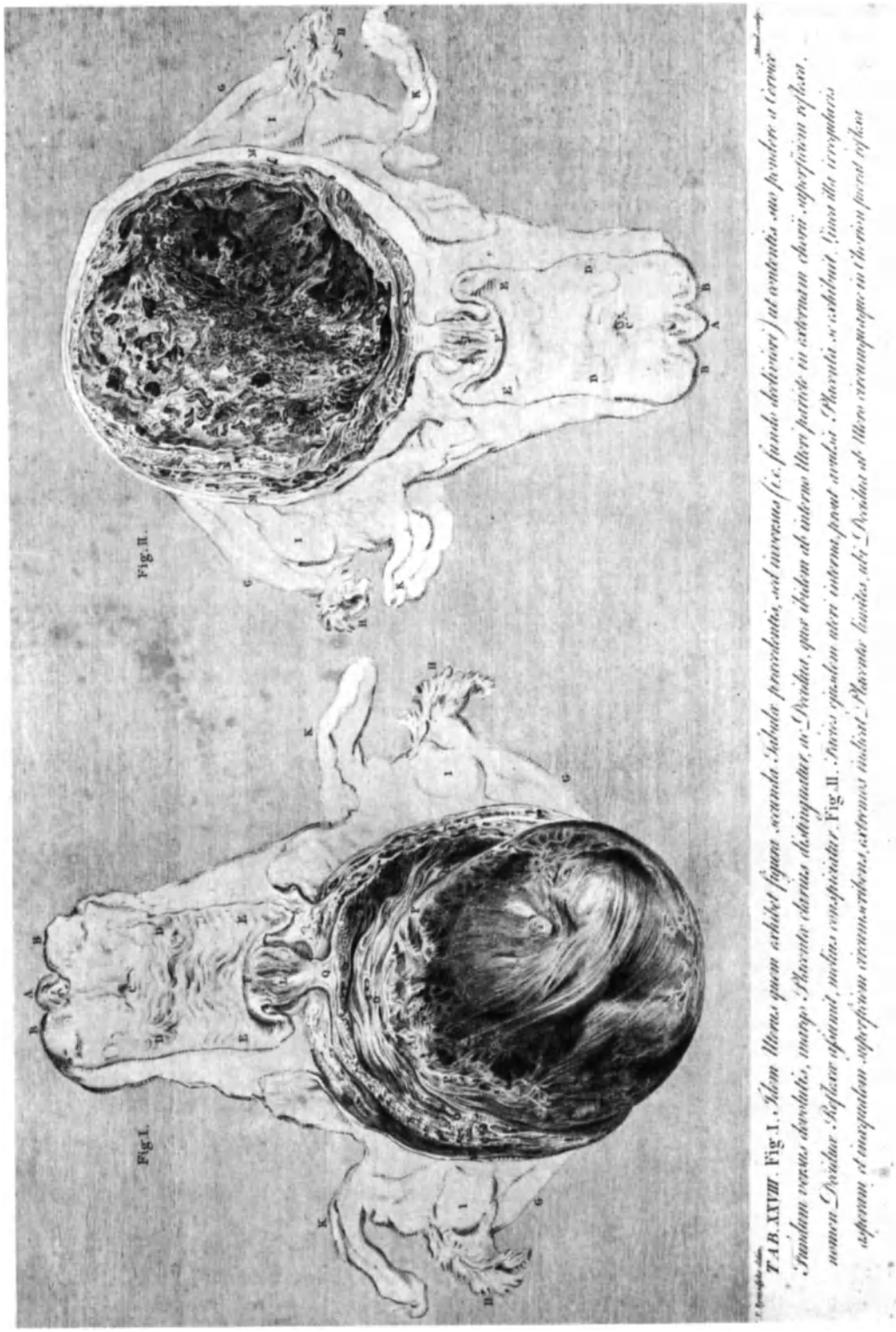

Figure 3.9 A plate from *Hunter's Anatomy of the Human Gravid Uterus* published in 1777 showing the decidual membrane.

shown not to be the sole preserve of the human placenta. In 1878 Creighton demonstrated their presence in the guinea pig placenta, and this was soon followed by their demonstration in other rodents (mouse, Tafani, 1886; rat, Duval, 1891), although we now know that they are an exclusive type of cell – most species manage without them. Apart from their size, another very characteristic feature of decidual cells, the presence of glycogen, was demonstrated before the end of the nineteenth century (Langhans, 1890).

As the cells appeared to be very unusual, there was obviously a lot of interest in their origin. Henning (1872) considered them to be enlarged and modified leucocytes, whilst Overlach (1885) and Frommel (1883) described them as modified glandular cells. However, their true origin from stromal fibroblasts was first suggested by Creighton (1878) from studies on the guinea pig placenta. The resemblance of the decidua to granulation tissue had been noted by Turner in 1876, who thought that it might have a similar defensive function. This theme was expanded by Fothergill (1899).

> The fertilized ovum, in arranging for its own nutrition, is known to make an attack on the maternal structures. The decidual cell, it is suggested, has been evolved as a protection, its function being to prevent injurious invasion of the uterine wall by the fetal elements of the placenta.

In the same paper Fothergill also makes the important statement 'the formation of a decidual membrane is now thought by some to be possible apart from pregnancy either uterine or ectopic'. The observation that a decidual reaction takes place in the uterus in cases of ectopic pregnancy had been noted earlier and had been commented upon by Turner in 1873. It was in fact a very interesting observation as it could only have been made in women. Apart from the fact that other animals rarely, if ever, have ectopic pregnancies it is only in women that decidual transformation occurs in the absence of a stimulus to the wall of the endometrium, which is normally of course an implanting blastocyst. This was apparently puzzling Marshall (1910) when he wrote the first edition of this book. Discussing decidual cells in the text he says 'the first appearance in the superficial layers of the mucosa has suggested a stimulus for their formation arising from the products of conception' but he adds a footnote:

> Under abnormal conditions the formation of decidual cells occurs even though no ovum is present in the uterus, e.g. in tubal pregnancy in the human female. Whether this indicates a chemical stimulus from the ovum, or perhaps from the corpus luteum, effected through the blood stream, is not yet known.

Here, before the isolation of the hormones of the ovary, we see one of the first suggestions that there might be hormonal control of decidual transformation and the first indication of the difference between the stimulus for the formation of the decidua in rodents and women.

At about the same time that Marshall was writing his book two very important discoveries were made by Leo Loeb (1907, 1908a), a pathologist working in America. He, like earlier workers, had been impressed by the similarity of the decidual response in rodents to the granulation tissue response following injury in other connective tissues. He therefore investigated the reaction of the guinea pig uterus to injury. However, he had the foresight, presumably influenced by the work on oestrous cycles by Walter Heape (1900), to injure the uterus on various days of the oestrous cycle. He obtained a decidual response to the trauma, but only when the uterus was injured at certain times. Thus, in one series of experiments he made two very important discoveries: first that the rodent uterus would respond to trauma with the formation of a decidual cell reaction and sec-

ondly that the responsiveness of the uterus varied during the cycle. This was later confirmed by W.M. Allen (1931) in the rat and Courrier and Kehl (1930) in the rabbit. [As Loeb (1923) was also the first to demonstrate the influence of the uterus on the corpus luteum he must surely rank as one of the greatest uterine physiologists.]

Loeb likened his trauma-induced deciduomata to tumours of decidual tissue which had already been reported in women (Ladinski, 1902) and which were known as **deciduomas malignum**. The name deciduoma has stuck for artificially induced decidual tissue although they are the antithesis of tumours, showing highly organized and controlled growth and programmed cell death. They are sometimes referred to as **placentomas**.

Loeb's discovery of the traumatic decidual cell reaction in guinea pigs was soon followed by its demonstration in other rodents (rat, rabbit, and dog, Loeb, 1908b; Frank, 1911; Gasbarrini, 1911; Kranz, 1914), and in 1922 Long and Evans introduced what they considered to be a more physiological method of decidual induction – a silk thread placed in the uterine lumen.

The end of the nineteenth and first third of the twentieth century saw the beginnings of reproductive endocrinology starting with the discovery of the endocrine functions of the ovarian follicle (Lataste, 1887) and corpus luteum (Beard, 1897; Prenant, 1898; Fraenkel and Cohn, 1901; W.B. Bell and Hicks, 1909) and the demonstration of the correlation of the menstrual cycle with ovarian activity (Schroder, 1915). This culminated in the isolation of oestradiol (E. Allen and Doisy, 1923) and progesterone (W.M. Allen and Wintersteiner, 1934; Slotta *et al.*, 1934; Butenandt and Westphal, 1934). The role of the anterior pituitary in the control of the ovary and the importance of copulation for the maintenance of the corpus luteum in rats was also discovered during this period (Brouha, 1928). A useful technological advance at the end of this period was the discovery by Shelesnyak (1931) that pseudopregnancy could be induced in rats by electrical stimulation of the cervix. This is not possible in the mouse.

By the end of this period it had been established that both ovarian hormones in succession are required for decidualization (Corner and Warren, 1919; Weichert, 1928). This was summed up by Nelson and Pfiffner (1930):

> The conditions essential for placentoma formation are (1) the uterus must be prepared for the luteal stimulus by the action of the follicular hormone upon the endometrium – a priming action, (2) the uterus must be sensitized by progesterone for several days prior to stimulation and must be under its influence throughout the life of the decidua.

Soon after the elucidation of the hormonal involvement in the regulation of the decidual cell reaction two classic descriptions of the histology of the decidual cell reaction were published (Selye and McKeown, 1935; Krehbiel, 1937). These publications showed clearly that the decidua is a complex structure which forms during several days following implantation (Figure 3.10).

In their descriptions the authors showed that the decidua contained three primary cell types:

1. the antimesometrial cells which form first, in close association with the implanting blastocyst,
2. the mesometrial decidual cells, which form later around the lateral and mesometrial aspects of the lumen and
3. granulated cells which form in the mesometrium, the metrial gland cells.

Antimesometrial decidual cells are large basophilic cells containing two or more nuclei with many mitochondria, abundant rough endoplasmic reticulum and Golgi complexes, while the mesometrial cells are smaller, irregular in shape, close packed, and usually

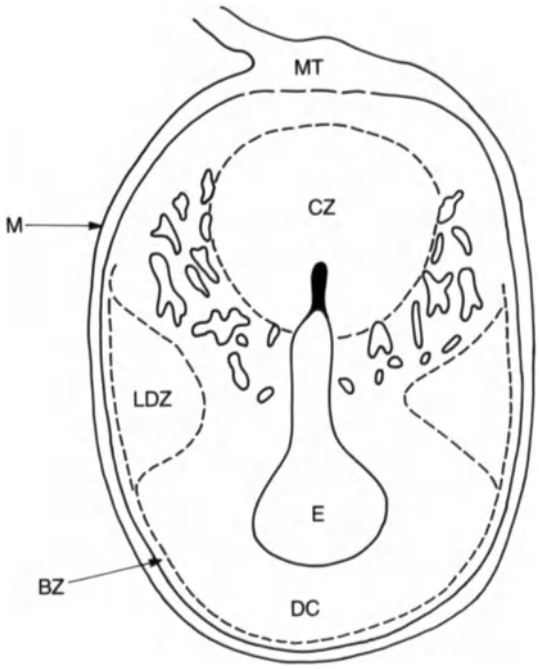

Figure 3.10 Diagrammatic representation of transverse sections of implantation sites on day 8½ of pregnancy in the mouse. Implantation of the embryo (E) occurs in the antimesometrial side of the lumen. The antimesometrial decidua forms the decidua capsularis (DC). The untransformed region of the stroma is termed the basal zone (BZ). The mesometrial decidua is composed of the central zone (CZ) and the lateral sinusoids (LS). The lateral decidual zone (LDZ) is formed between the major decidual ones. The metrial gland develops in a restricted region of the myometrium (M), the mesometrial triangle (MT) through which maternal blood vessels gain access to the uterus. (From S.C. Bell, 1983a, by permission of the editor, *Oxford Reviews of Reproductive Biology*.)

have a single nucleus and much glycogen. The third type of cell, the granulated metrial gland cell, was clearly described by Selye and McKeown (1935). The major development of these cells does not occur until later than the first two types of cell. Similar granulated cells had already been described in the human uterus during pregnancy (Weill, 1921), although the relationship of metrial gland cells to these was not appreciated at the time. These early workers also described the formation of large vascular sinuses laterally to the implanting blastocyst, indicating the importance of vascular changes.

It is apparent that by about 50 years ago many of the fundamental facts about the decidua were known. Progress since then has been partly in response to advances in technology, for example the development of the electron microscope, autoradiography, histochemistry and the availability of drugs affecting cellular metabolism, and partly due to the conceptual advances in other aspects of cell biology, especially concepts about local hormones and cell control mechanisms. Whereas the emphasis for the first hundred years after Hunter's death had centred on the human decidua, most of the emphasis over the last 50 years had centred on the decidua of experimental animals, especially rats and mice.

3.5.2 THE ORIGIN OF DECIDUAL CELLS

The problem of the origin of decidual cells, which had caused much controversy in the later nineteenth century, was settled beyond reasonable doubt with respect to the antimesometrial and mesometrial decidual cells using the technique of autoradiography. Galassi (1968) labelled stromal cells during the S phase of mitosis with radioactive thymidine and found that the label appeared in the nuclei of the decidual cells which formed subsequently, thus confirming Creighton's original suggestion. The suggestion of Kearns and Lala (1982) that the decidual cells derive from the bone marrow has been challenged by several workers (Gambel *et al.*, 1985; S.C. Bell, 1983a).

(a) Metrial gland

The origin of the metrial gland cells was not so easily settled. The early workers considered they formed from mesometrial mesenchymal cells (Selye and McKeown, 1935;

Baker, 1948), from the trophoblast (A.D. Dickson and Bulmer, 1961) or stromal fibroblasts (Larkin and Schultz, 1968; Larkin and Cardell, 1971). The first suggestion of what is now considered to be their true origin was by L.J. Smith (1966), who proposed that they were transformed from lymphocyte-like precursor cells. This suggestion was based on light microscope studies but supported later from examination with the electron microscope (I. Stewart and Peel, 1977, 1978). More recent work using bone marrow chimeras between rats and mice has confirmed that the metrial gland cells are derived from cells originating in the bone marrow (Peel *et al.*, 1983). A similar origin for endometrial granulocytes in the human uterus has been demonstrated (J.N. Bulmer and Sunderland, 1983; J.N. Bulmer *et al.*, 1987). The presence of immunoglobulin in the cytoplasm of metrial gland cells (D. Bulmer and Peel, 1977; Mitchell *et al.*, 1980) adds further weight to a lymphocyte origin.

3.5.3 THE MORPHOLOGY OF THE DECIDUA

(a) Resident decidual cell

There have been many detailed histological descriptions of the decidua (both natural and artificial) in several species (Finn and Hinchliffe, 1965; DeFeo, 1967; S.C. Bell, 1983a) since the classic papers by Selye and McKeown (1935) and Krehbiel (1937). The use of Feulgen straining and the realization of the importance of DNA in the nucleus of the antimesometrial decidual cell led to the finding that the decidual cell, described by Turner as being colossal, is polyploid (Sachs and Shelesnyak, 1955) and very often binucleate. Using autoradiography, it has been shown that the nucleus of the antimesometrial decidual cell can have up to 64 times the haploid DNA content and arises by a process of endoreduplication, whilst multinucleate cells arise from mitoses without subsequent cell division (Zybina and Grishenko, 1972; Leroy *et al.*, 1974a; Ansell *et al.*, 1974). The mesometrial decidual cells are somewhat smaller but still polyploid.

The decidual cell has also been shown to acquire the ability to synthesize specific enzymes, such as alkaline phosphatase (Finn and Hinchliffe, 1964) and ornithine decarboxylase (Collawn *et al.*, 1981).

With the electron microscope a characteristic relationship between the decidual cells has been demonstrated (Finn and Lawn, 1967; Kleinfeld *et al.*, 1976). The cells send out processes which make contact with adjacent cells and very often send finger-like processes into the cell. The area of contact between cells is characterized by gap junctions. Similar gap junctions have been shown between the predecidual cells which are formed during the menstrual cycle in women, although in later pregnancy the decidual cells are separated from each other by a connective tissue matrix (Lawn *et al.*, 1971). Gap junctions are now known to be formed of connexins, and high expression of these is found in developing decidual cells (Winterhager *et al.*, 1991). Intercellular junctions are thought to be important in the flow of material from one cell to another, especially in tissue growth and differentiation (Lowenstein and Penn, 1967). Decidual cells have also been shown to contain large quantities of intermediate fibres, made of two different proteins – vimentin and desmin. The former is also present in non-decidualized stromal cells, whereas the latter accumulates predominantly after decidualization (Glasser and Julian, 1986).

In the early stages of decidualization the stromal cells around the periphery of the decidua are undergoing active division (Finn and Martin, 1967). This is in response to the decidual stimulus.

(b) Extracellular matrix

The extracellular matrix surrounding the developing decidual cells changes, especially

with regard to hyaluronic acid (Brown and Papaioannou, 1992), collagen (Kisalus *et al.*, 1987; Karkavelas *et al.*, 1988), laminin (Wewer *et al.*, 1986) and fibronectin (Grinnell *et al.*, 1982).

Hyaluronic acid disappears from the extracellular matrix as decidualization progresses (Brown and Papaioannou, 1992), and the amount of collagen is reduced considerably (Myers *et al.*, 1990), with a change to predominantly non-fibrillar type IV collagen (Aplin *et al.*, 1988) and loss of collagen type VI (Mulholland *et al.*, 1992).

Decidual cells, however, retain their ability to synthesize collagen (Oliveira *et al.*, 1991), and the diameter of collagen fibres increases with decidualization (Alberto-Rincon *et al.*, 1989). At the same time decidual cells take up collagen (Zorn *et al.*, 1989), thus contributing to the remodelling of the stroma.

(c) Vascular changes

An important early response to a blastocyst noted by the earlier workers was oedema of the stroma. Extravasation of fluid into the tissues is an early response to an inflammatory stimulus and is caused by increased permeability of the blood vessels supplying the area. This can be demonstrated by injecting pontamine sky blue or some other dye into the bloodstream. The dye binds to albumin and normally therefore remains in the vessels. Blue staining of the tissues indicates increased vascular permeability. By injecting the dye into rats over the first few days of pregnancy Psychoyos (1960, 1961) showed that an increase in vascular permeability was a very early response of the uterine stroma to a blastocyst. The injection of ^{125}I-labelled human serum has since been used to give a more accurate picture of changes in vascular permeability (Milligan and Mirembe, 1984). The dye reaction has proved to be a very important finding, not only for its inherent interest but also because it makes it possible to delineate areas of the uterus which are responding to a decidualizing stimulus (for example an implanting blastocyst) well before any change in the uterus can be seen macroscopically. It is interesting to quote the comments of Emrys-Roberts (1909), one of the earliest investigators of implantation in the guinea pig:

> serial sections were cut from one end to another. This was necessarily a laborious and trying undertaking; each individual section had to be examined since there was no macroscopic indication as to the site of the embryo. Indeed it happened that many thousands of sections have been examined fruitlessly.

(By marking out areas with the dye much fruitless work is avoided!).

Several other species also undergo the dye reaction, and it is likely that it is of general applicability (rabbit, Hoos and Hoffman, 1980; mouse, Finn and McLaren, 1967; Milligan, 1987; guinea pig, Orsini and Donovan, 1971; ewe, Boshier, 1970; sow, Keys and King, 1985, 1988; ferret, Mead *et al.*, 1988). [The dye reaction to injected oil will, however, occur in the uteri of animals which have not been sensitized for decidualization (Milligan and Mirembe, 1985), indicating that although it is a prerequisite for decidualization it is not exclusive to it.] Abrahamsohn *et al.* (1983) and Hoffman and Hoos (1984) have shown ultrastructural changes in the relevant blood vessels, including fenestrations in the endothelial cells and gaps between them.

Oedema occurs in the stroma of the human and monkey endometrium during the menstrual cycle, probably associated with a change in the permeability of the blood vessels. This does not depend on a stimulus from the blastocyst. Further increase in vascularity does however occur in the human following implantation (Bouda, 1969).

Another interesting vascular effect is the absence of a basement membrane around the large endothelial cells which form the large sinusoids in the lateral decidua (Hall, 1968,

1969). Associated with this is the disappearance of the enzyme adenosine triphosphatase (Hall, 1968). The vascular changes in the rodent uterus during early pregnancy have been studied using the transmission electron microscope (Christofferson and Nilsson, 1988) and scanning electron microscope examination of corrosion casts (Takemori *et al.*, 1984). It has been shown that the area around the implanting blastocyst (the primary decidual zone) is free of blood vessels (Rogers *et al.*, 1982a,b) and may provide a partial permeability barrier (M.B. Parr and Parr, 1989). This has been confirmed by later work (Tawia and Rogers, 1992) using an *in vivo* microscopy technique in rats. At the time of implantation an avascular area surrounded by large-diameter vessels in which the flow was sluggish was demonstrated. Leucocyte rolling and adhesion was also a feature of these vessels, which fits well with the finding of increased numbers of leucocytes in the stroma at that time (Finn and Pope, 1991).

(d) Metrial gland

The granulated cells of the metrial gland which form in the metrial triangle in rodents have been extensively studied over the last 50 years (B.L. Baker, 1948; Wislocki *et al.*, 1957; A.D. Dickson and Bulmer, 1961; L.J. Smith, 1966; Larkin and Schultz, 1968; Larkin and Flickinger, 1969; Peel and Bulmer, 1977; I. Stewart and Peel, 1977; Bulmer *et al.*, 1983; Peel, 1989) as have the similar granulated cells in the uterine stroma of the human and rhesus monkey (Cleveland, 1941; Hamper, 1955; Dallenbach-Hellweg, 1967; Cardell *et al.*, 1969; Asplund and Holmgren, 1974; J.N. Bulmer *et al.*, 1980). As with the formation of oedema and decidual cells, primate granulated cells appear in the endometrium towards the end of the menstrual cycle and are not dependent for their formation on the presence of a blastocyst. Furthermore, they are scattered throughout the stroma.

Metrial gland cells are characterized by having numerous diastase-resistant PAS-granules in the cytoplasm. By day 12 in the rat they form a mass of cells in the mesometrial triangle. The cells have only been studied in detail in the rat and mouse, where rather surprisingly they differ in size of cell and size of granule. The mouse cells and their granules are much larger (Peel and Bulmer, 1977), although the rat cells have more granules (I. Stewart and Peel, 1977). The granules have an electron-dense amorphous matrix enclosed by a close-fitting membrane, and it has been suggested they are lysosomes (Peach and Bulmer, 1965). This is supported by the localization of acid phosphatase, leucine aminopeptidase and nitrocatechol sulphatase in the granules (D. Bulmer, 1968).

In addition to the three main cell types described and the blood vessels, other cells have recently been delineated by the use of cell-surface markers (Bernard *et al.*, 1978; S.C. Bell, 1985). The best defined are the Fc receptor-bearing cells, which are probably macrophages (G.W. Wood, 1980; Kirkwood, 1981). They also bear Ia antigen (Searle *et al.*, 1983). These are also found in non-decidualized uteri (Kirkwood, 1981), but there is a higher number in the decidualized uterus (Rachman *et al.*, 1981). They are probably an infiltrating cell (S.C. Bell, 1985). Fc receptor-bearing cells have also been reported in the metrial gland (Bray *et al.*, 1978; Bray and Craggs, 1979).

3.5.4 BIOCHEMICAL CHANGES

Obviously the morphological changes in the stroma will be accompanied by biochemical changes. For a detailed account see S.C. Bell (1983a,b).

(a) Nucleic acids

Any tissue undergoing cell division will be increasing its content of DNA (Ledford *et al.*, 1978) and RNA (Miller *et al.*, 1968; Jayatilak *et al.*, 1989) and of course the reverse is true for

tissues undergoing cell death. Equally, tissues undergoing active differentiation will most likely register changes in RNA and protein content (Lejeune *et al.*, 1982). Actinomycin D halts decidualization in rats (Burin and Sartor, 1965; Sennanes and Psychoyos, 1970) and mice (Finn and Martin, 1972b), suggesting that transcription and translation are taking place. Changes in RNA content of the uterus during decidualization have been among the earliest changes recorded (Heald *et al.*, 1970; Glasser, 1972; Miller, 1973). Most of the change is due to increased ribosomal RNA synthesis (Heald *et al.*, 1972). More detailed analysis has revealed changes in the species of RNA synthesized (Heald and O'Hare, 1973; O'Grady *et al.*, 1975; Glasser and Clark, 1975). S.C. Bell (1983a) pointed out that as some of these changes occur very early in the decidual reaction they may represent the onset of rapid cell division following the initiation of decidualization. It would nevertheless be very surprising if the complex differentiative changes of decidualization were not controlled to a large extent by changes in types of RNA synthesized.

(b) Enzymes

Changes in the synthesis of several enzymes have been demonstrated. Associated with increased ribosomal RNA synthesis in the early stages of decidualization there is an increase in RNA polymerase I activity (Serra *et al.*, 1978). Another enzyme which is probably associated with RNA synthesis is ornithine decarboxylase. This is involved in the production of polyamines, which are thought to be involved in the stabilization of ribosomes, the stimulation of RNA synthesis and inhibition of RNA degradation (Tabor and Tabor, 1972). Increased activity of ornithine decarboxylase has been demonstrated in the uterus in the early stages of decidua formation either during pregnancy (Saunderson and Heald, 1974) or after the artificial induction of decidualization (Heald, 1979; Collawn *et al.*, 1981). However, Milligan *et al.* (1992) have found that the enzyme increases in the uterus in response to stimuli not causing a decidual reaction and they question an association with decidualization.

Amine oxidase has been demonstrated in the decidual portion of the human placenta, and it has been suggested that it may fulfil a maternal protective role in neutralizing high levels of polyamines derived from the feto-placental unit (see S.C. Bell, 1986).

Lysosomes contain several proteolytic enzymes. These are important in the breakdown and remodelling of tissues. During the stage when decidual tissue is growing there is a decrease in some lysosomal enzymes (acid cathepsin D: J.G. Wood and Barley, 1970; Moulton, 1974; Moulton and Ingle, 1981), with little change in others (β-glucuronidase, Moulton, 1974; Jelinek and Jelinkova, 1977; J.G. Wood and Barley, 1970). During decidual regression, mostly investigated using the artificially induced decidual reaction, there is a dramatic rise in lysosomal enzymes (Lobel *et al.*, 1965; J.G. Wood and Barley, 1970).

Whilst changes in the enzymes so far discussed can be fairly easily understood in terms of what is known to be happening during decidual growth and regression, the reason for the appearance of the enzyme alkaline phosphatase in the decidua is at present unknown. The presence of this enzyme was first shown histochemically during implantation and after oil induction of decidualization in mice (Finn and Hinchliffe, 1964). It first appears soon after the pont-amine sky blue reaction, coincidental with decidual transformation of the stromal cells (Finn and McLaren, 1967) and disappears when regression of the tissue has commenced (Hall, 1969). *In vitro* and *in vivo* the presence of the enzyme is enhanced by prostaglandin E_2 (S.A.J. Daniel and Kennedy 1987; Yee and Kennedy, 1988). It belongs to the bone–liver–kidney class of alkaline phosphatase (J.W. Pollard *et al.*, 1990).

Alkaline phosphatase has also been demonstrated biochemically in the decidua of rats and mice (Manning *et al.*, 1966) and histochemically in the predecidual cells in the human uterus (E.W. Wilson, 1969). The role, if any, played by the enzyme in the decidua is unknown, although there have been suggestions (R.N. Murdoch *et al.*, 1978). Alkaline phosphatase is also found in the embryo in association with differentiating tissues, and it has been suggested that it is in some way involved in the differentiation of cells.

Other enzymes which have been demonstrated in decidual cells but to which no function can be assigned are adenosine deaminase (Hong *et al.*, 1991; Knudson *et al.*, 1991) and renin (K.J. Shaw *et al.*, 1989). The latter is involved in salt and water balance when produced by the kidney but its functional significance in the decidua is unknown.

(c) Polypeptides and proteins

The possible functions of the decidua involve actions on tissues or organs remote from the implantation site. It is not surprising therefore to find that the secretion of proteins and smaller polypeptides by the decidua is an active area of study (see S.C. Bell, 1983a, 1985 for reviews).

For both the human and rodent, evidence is accumulating that decidual cells secrete a protein with prolactin-like properties (human, Riddick and Kusmik, 1977; Golander *et al.*, 1978; Tomita *et al.*, 1982; rat, Gibori *et al.*, 1974; Basuray and Gibori, 1980; Terranova, 1980). In women decidual tissue, whether obtained in the late luteal stage of the menstrual cycle or in pregnancy, contains prolactin. It is likely that the prolactin in amniotic fluid arises from the decidua and it has been suggested that it is related to osmoregulation across the amnion (Tomita *et al.*, 1982).

Rodents require an additional source of luteotrophin in the second part of pregnancy to maintain the corpus luteum, and it is likely that decidual prolactin supplies this. Pseudopregnancy is prolonged in the rat when deciduomata are present in the uterus (Peckham and Greene, 1948).

Many other polypeptides have been found to be secreted during the early stages of pregnancy. Some of these are associated with the differentiation of the stroma into the decidua, e.g. basement membrane materials, laminin, fibronectin, type IV collagen (Wewer *et al.*, 1986), whereas others appear to be a response of the uterus to the hormones of pregnancy (Adams *et al.*, 1981; Waites and Bell, 1984, 1989; Bell, 1985; Nieder and Macon, 1987). A particularly interesting recent finding is that the human decidualized endometrium secretes pregnancy associated endometrial α_1-globulin, which is an insulin-like growth factor binding protein. It is suggested that it may be involved in trophoblast invasion of the endometrium (S.C. Bell *et al.*, 1988).

An interesting protein which has been found in the plasma during the late secretory phase of the human menstrual cycle and early pregnancy (Bischof, 1981) is pregnancy-associated plasma protein A (PAPP-A). This is probably secreted by the decidua (Schindler *et al.*, 1984). It has been suggested that PAPP-A is a pregnancy-associated analogue of normal human α_2-macroglobulin. Bischof *et al.* (1984) speculate that PAPP-A, by its inhibitory effect on C3, reduces complement-mediated lysis and this contributes to survival of the fetal allograph. Similarly, a protein found in rat decidual tissue (decidualization-associated protein, DAP) is immunochemically identical to serum α_2-macroglobulin (S.C. Bell, 1979a,b). This protein is an acute-phase reactant (Koj, 1974) which is synthesized by the liver in response to inflammatory stimuli. Pregnancy-associated α_2-macroglobulin has been demonstrated in the serum of normal pregnant women (Stimson *et al.*, 1983) (see Waites and Bell, 1986, for a discussion of the pregnancy proteins and the acute-phase response).

There are many studies on decidual proteins being carried out at present, and with the continuing development of protein chemistry major advances can be expected in the next few years (Nieder, 1988; Weitlauf and Suda-Hartman, 1988; S.C. Bell *et al.*, 1989).

3.5.5 HORMONAL CONTROL OF DECIDUALIZATION

Decidualization is normally part of implantation, and in preparing the uterus for implantation the ovarian hormones also prepare the endometrium to respond to an artificial decidual stimulus. Changes in receptors for oestrogens and progesterone have been demonstrated in the uterus during decidualization (Martel *et al.*, 1984). Not surprisingly, animals which do not require nidatory oestrogen for implantation do not require it for decidualization (guinea pig, Deanesly, 1960). It is also found that more traumatic stimuli such as crushing can elicit a decidual cell reaction (DCR) in the rat (Rothchild and Meyer, 1942) and mouse prepared only with progesterone, although to obtain a response to intraluminal inducers, such as oil, nidatory oestrogen is necessary (Finn, 1965, 1966a). Once established, the decidual reaction including the metrial gland (Martel *et al.*, 1989) is dependent on a supply of progesterone. However, even with a continuous availability of progesterone, the decidua has a predetermined lifespan in the mouse and rat and regresses after 6 or 7 days.

The pituitary hormones of course control the secretion of the ovarian hormones and are thus critical in decidualization. There is some evidence that as well as the gonadotrophins, growth hormone and thyroxine are also involved directly in decidualization (Kennedy and Doktorcik, 1988).

In women the situation is different. Decidualization occurs during the menstrual cycle (Novak and Te Linde, 1924) and requires only ovarian hormones for its induction. Whether both progesterone and oestrogen are necessary is not known but once started, the decidua will continue to grow with progestin treatment only, and the decidual tissue is maintained as long as a source of progesterone is available (Durham, 1961; Eichner *et al.*, 1951).

3.5.6 MEDIATORS OF THE DECIDUAL RESPONSE

In spite of much work on the nature of the stimulus for the induction of decidualization (DeFeo, 1962) there is at present no understanding of the exact mechanism whereby the presence of a blastocyst triggers off the events leading to decidualization. The fact that several artificial stimuli can elicit the reaction possibly suggests that the triggering involves some fairly simple physical reaction between the contacting surfaces of the trophoblast and uterine epithelium, rather than the passage of a chemical substance from the blastocyst. Although Weitlauf (1989) has suggested that the mouse blastocyst may release factors which influence the uterus in the peri-implantation period, this has not been confirmed.

Stimuli which overtly damage the uterus, such as crushing, scratching or the insertion of a thread through the wall, are unlikely to mimic closely the presence of a blastocyst in the rat or mouse, which does not appear (from light or electron microscopic examination) to be associated with damage to the endometrium. This may not however be true for animals with interstitial implantation, such as the guinea pig, in which the blastocyst burrows actively into the endometrium. It is interesting that trauma but not oil or beads is an effective decidual stimulant in the guinea pig (Blandau, 1949) and baboon (Wheeler *et al.*, 1983). Furthermore, the endocrinological conditions necessary for the traumatic deciduoma in the rat and mouse are not identical to those necessary for implantation (Finn, 1965).

It would be expected that the instillation of

substances into the lumen of the uterus would mimic more closely the presence of a blastocyst. They are acting at the same site as the blastocyst, and require the same hormonal conditions as implantation to be effective. The chemical nature of the substances does not appear to be important. Among the most effective intraluminal inducers are various vegetable and mineral oils (including the non-toxic fluorocarbon oil, Flutec (Milligan, 1987), solutions of carrageenan and agar and air (Finn and Keen, 1962a; Orsini, 1963). It is also important to recognize that the injection of a substance into the lumen may not be mimicking the initial stimulus. The substance may pass through the surface membrane of the luminal epithelial cells and activate later stages in the message transmission process. For example, the injection of phorbol esters and calcium ionophores into the uterine lumen induces a decidual reaction (Kyd and Murdoch 1992), presumably indicating that the decidual stimulus activates the phosphatidylinositol pathway within the cell.

It is tempting to try to find a common factor associated with all the decidual stimuli. It has been suggested that the activation of Hageman factor (factor XII) may be a common attribute (Finn, 1986). This factor has a central role in the inflammatory response, blood clotting and the complement system. It is activated by the presence of negative ions, which in injury are provided by exposed collagen. Carrageenan is known to provide negatively charged particles (Warren and Kellermeyer, 1968) and the blastocyst has negative charge on its surface (Clemetson *et al.*, 1970; Nilsson *et al.*, 1974). Possibly oil also acts in a similar way.

Whatever the initial trigger, its effect will be transmitted by the luminal epithelium (Lejeune *et al.*, 1981) and will be followed by a chain of chemical changes in the epithelium and stroma leading to the various vascular and cellular events of decidualization. Possibly traumatization of the uterus activates the chain after the initial reaction at the luminal surface. As the traumatic decidual stimulus does not depend on luteal-phase oestrogen, it suggests that the changes induced by luteal-phase oestrogen on the luminal cell surface (causing the second stage of closure) in some way allows triggering of the decidual cell reaction by the blastocyst or oil.

Several substances reviewed here have been implicated in the propagation of the decidual reaction once triggered, and it is interesting that most of these are substances involved in inflammation (Solomkin and Simmons, 1983).

(a) Histamine

A possible role for histamine in the stimulation of the decidual cell reaction was put forward by Shelesnyak (1957). He and his coworkers published evidence that a decidual cell reaction could be stimulated by the intraluminal injection of solutions of histamine and prevented by the administration of antihistamines (Shelesnyak, 1960). The evidence, however, has been challenged by later workers, who were unable to confirm that intraluminal histamine caused a reaction (Finn and Keen, 1962; Banik and Ketchell, 1964) or that classical antihistamines blocked either implantation or the decidual reaction (Finn and Keen, 1962c) when injected systemically. When injected intraluminally antihistamines caused such profound disruption of cellular integrity that any effect on implantation may not be associated with an effect of histamine (Tachi *et al.*, 1970). Several workers have also shown a change in the mast cell population in the uterus at the time of implantation (Gibbons and Chang, 1972; Brandon and Bibby, 1979), and as these cells release histamine (Archer, 1959) this was taken as evidence for a role for histamine in implantation. However, the finding that the uteri of mast cell-deficient mice decidualize normally (Hatanaka *et al.*, 1982; Wordinger *et al.*, 1986) makes it unlikely that products of

these cells play an essential part in eliciting the decidual cell reaction, although it does not rule out a role for histamine from other cells.

Since the discovery of H_2 receptors and drugs which block them, interest has been renewed in a possible role for histamine, especially in the elicitation of the vascular response (Brandon and Wallis, 1977; Dey *et al.*, 1979) in association with other mediators. In support of this, it is suggested that the enzyme histamine decarboxylase may be present in the endothelium of small uterine blood vessels (Psychoyos and Martel, 1985).

(b) Prostaglandins

There is little doubt that prostaglandins and possibly leukotrienes (Cejic and Kennedy, 1991) are involved in the development of the decidual cell reaction, and many relevant studies have been published over the last two decades (Hoffman and Strong, 1976; Tobert, 1976). Their exact role in decidualization is not however settled. It is very likely that they play some role in vascular changes (T.J. Williams, 1979; Kennedy, 1979; Psychoyos and Martel, 1985) and may promote fibroblast proliferation (Hial *et al.*, 1977) and alkaline phosphatase activity (Daniel and Kennedy, 1987). Prostaglandin antagonists such as indomethacin and tranyccypromine prevent implantation and the artificially induced decidual cell reaction in several species (Castracane *et al.*, 1974; Tobert, 1976; Saksena *et al.*, 1976; Hoffman, 1978; Rankin *et al.*, 1979), as do leukotriene inhibitors in rats (Tawfik and Dey, 1988).

Concentrations of various prostaglandins increase in areas of the uterus which have been stimulated to decidualize, e.g. PGE_2 (Hoffman *et al.*, 1977; Kennedy, 1977; Evans and Kennedy, 1978; Jonsson *et al.*, 1979), PGF_2 (Saksena *et al.*, 1974; Milligan and Lytton, 1983), PGI_2 (Kennedy and Samecnik, 1978; Phillips and Poyser, 1981).

Decidual cells synthesize prostaglandins, principally PGE_2 (Anteby *et al.*, 1975; K.I. Williams and Downing, 1977; Tsang and Ooi, 1982), and the uterine stroma acquires receptor for PGE_2 when under the hormonal conditions which induce sensitivity for decidualization (Kennedy *et al.*, 1983; Psychoyos and Martel, 1985). It is suggested that the uterine epithelium releases prostaglandin, which passes to the stroma, causing decidualization; the decidual cells so produced then produce more prostaglandin to propagate the response. They are presumably involved in the signalling of decidual transformation throughout the process (Kennedy, 1985), but are probably not the initial stimulus.

(c) Cyclic AMP

Prostaglandins increase the concentration of cyclic AMP (cAMP) in many cells, so it is not surprising that there is a rapid rise in levels of the nucleotide after decidual induction (Leroy *et al.*, 1974b; Swift and O'Grady, 1976; Rankin *et al.*, 1977; Kennedy, 1983). Attempts to induce implantation in ovariectomized rats and mice with dibutyryl cAMP have given contradictory results, some workers claiming a positive effect (Webb, 1975; Holmes and Bergstrom, 1975) and others no effect (Wu and Chang, 1977). Fernandez-Noval and Leroy (1978) were able to induce implantation with non-cyclic AMP.

Adenylate cyclase, the enzyme responsible for the formation of cAMP, is activated after the application of a decidual stimulus in the rat (Sanders *et al.*, 1986; Yee and Kennedy, 1991), and alloxan, an inhibitor of adenylate cyclase, inhibits implantation. There is also alteration of adenylate cyclase activity in the rabbit endometrium during pseudopregnancy, suggesting an involvement of cAMP in the regulation of endometrial sensitivity (Fortier *et al.*, 1989).

Whilst the results from these experiments must be considered inconclusive, it is likely that cAMP is a component of the transfer of

information from the decidual stimulus to the differentiated cell.

(d) Platelet-activating factor (PAF)

PAF is a potent vasoactive agent which is involved in inflammation. In early pregnancy it is produced by mouse embryos and causes thrombocytopenia (O'Neill, 1985). It has also been demonstrated in the rabbit uterus during early pregnancy (Angle *et al.*, 1985). It has been suggested that it is involved in the signalling of the decidual cell reaction (Spinks and O'Neill, 1988). In view of the relationship of the decidual reaction to inflammation, this is a very attractive idea. However, whilst some workers have been able to obtain a decidual reaction with synthetic PAF (Acker *et al.*, 1989), others have not (Milligan and Finn, 1990). The latter authors were also not able to block implantation with PAF antagonists. At present therefore, it is not possible to be sure whether it is involved or not.

Other mediators which are involved in inflammation, such as bradykinin (DeFeo, 1962; Humphrey and Martin, 1968) and plasminogen activator (Rybo, 1966; Strickland *et al.*, 1976), have also been studied during decidualization and implantation, but at present a role for them has not been proved.

3.5.7 DECIDUAL RESPONSE OF AGED ANIMALS

As rodent females get older the size of their litters gets smaller until breeding ceases (Biggers *et al.*, 1962). This usually occurs well before the death of the mother. One of the main causes of this declining fecundity is failure of the uterus to maintain the embryos to term. This situation is different from that found in women, in whom the principal cause of reproductive senescence lies in the ovarian depletion of oocytes.

In the mouse, rat and bank vole the decidual response of the uterus to an artificial stimulus declines as the mother gets older (Finn 1966b; Shapiro and Talbert 1969; Nerquaye-Tetteh and Clarke, 1987), and this is correlated in mice with failure of the luminal closure reaction which occurs in young animals in response to ovarian hormones (Finn and Martin, 1969b). The importance of failure of decidualization in causing reduced reproductive output in aged females is discussed by Finn (1970).

3.5.8 DECIDUALIZATION AND MENSTRUATION

Menstruation is one of the most obvious signs of reproductive activity in women, and must have puzzled primitive man, long before its role in relation to pregnancy was known. The relationship of the monthly sanguinous discharge to childbearing was suggested as early as the seventh century when Isodore of Seville (see Grant, 1978) wrote:

> After many menstrual days, however, the semen is no longer germiable because there is no menstrual blood by which the ejaculate can be irrigated. Thus semen does not adhere to the female parts; lacking this power to adhere it is lost. Likewise thick semen also lacks the power of growth, being unable to mix with the female blood because of its own excessive thickness. This is why men or women become sterile; from excessive thickness of semen or blood, or from excessive thinness.

Isodore had also noted that 'women are the only menstrual animal'. Considering that the bitch, the animal closest to man in his habitat, does show occasional vaginal discharges of blood, this observation, although correct, is rather surprising. In fact, in Victorian times there was strong support for the idea that menstruation was analogous to pro-oestrous bleeding in dogs.

The loss of blood at menstruation was assumed by the early medical writers to serve

some useful function. They would have had no way of knowing the cellular make-up of the discharge but Isodore, nevertheless, appreciated that it was not ordinary blood:

> On contact with this gore, crops do not generate, wine goes sour, trees lose their fruit, iron is corrupted by rust, copper is blackened. Should dogs eat any of it they go mad. Even bituminous glue which is dissolved neither by rain nor by [strong] water, polluted by this gore, falls apart by itself.

Because of the occasional putrefying appearance, smell and pain it was assumed that the period was a method of detoxification of the blood poisoning encountered by women (Medvei, 1982). It was considered to be a way of ridding the body of excessive blood ('the menstrual flow is woman's superfluous blood, it is termed 'menstrual' menstrua, because of the phase of the light of the moon by which this flow comes about' – Isodore) and if women failed to menstruate then bleeding was thought to be necessary. Trotula of Salerno, in her eleventh-century book *The Diseases of Women*, gives precise details of the necessary procedure, apparently quoting the work of Galen:

> If the menses are lacking and the woman is thin, the vein under the inner arch of her foot, the internal saphenous, should be lanced. On the first day from one foot, on the following day the other foot – the blood to be drawn as the case demands.

For hundreds of years the correct relationship of menstruation to reproduction was not appreciated. In the middle of the nineteenth century the study of reproductive processes in animals was well under way. Man was now aware of his descent from other animals, and there was a strong move to extrapolate from other mammals to man. Pouchet in 1842 suggested that menstruation corresponded to the period of sexual excitement in other animals. This view seems to have been held by many of the Victorian biologists (Heape, 1900; Bryce and Teacher, 1908; Marshall, 1910), with a certainty characteristic of the age. Heape (1900) stated 'the homology of the process in the bitch with that already described for the monkey is absolutely certain'. Marshall (1910) in the first edition of this work was equally certain and wrote at length on the similarity of the period of menstruation in women to the period of heightened sexual activity in other mammals and concluded that it demonstrated the remains of a primitive oestrous cycle.

Medical writers of the time, however, were putting forward an alternative view, that menstruation 'is only a secondary process, a degeneration of the mucous membrane which from a failure of pregnancy has not been able to fulfil its purpose' (Grosser, 1910). Gynaecologists held that menstruation was unique to some primates and occurred between successive oestrous cycles, not in association with them (Hartman, 1932). This view, that menstruation is associated with failure of implantation, is now accepted.

Disorders of menstruation are common, and the process has been studied in considerable depth from the medical point of view (reviewed by Shaw and Roche, 1980). The problem of why women should bleed at the end of the cycle if implantation has not taken place, whereas most other animals do not, has however received less attention. Corner in 1923 conjectured that:

> In order to provide for the highly specialized embryonic implantation of primates with its opening of maternal blood vessels into the intervillous space the endometrial process is carried so far as even to cause bleeding into the tissues during the last days of the interval at the time during which the early embryo is to be implanted. The action of the embryo alters the latter part of the process so as to inhibit or limit the haemorrhage, but if no embryo be present to utilize the extravasation, then

> the blood escapes into the uterine lumen and visible bleeding occurs.

He goes on to suggest that this is the reason for the total absence of menstruation in species which have a less specialized form of implantation. More recently, Fox (1985) concluded that the reason women menstruate is 'the very rapid rate at which oestrogen and progesterone levels fall as the corpus luteum degenerate, in some species oestrogen and progesterone values decline at a much slower rate and menstruation is avoided although only at the cost of lengthening the menstrual cycle'. In his view 'menstruation is the price that women pay for shortening of their menstrual cycle which allows for a greater number of possible conceptions during their limited years of fertility'.

The latter very teleological view is not in accordance with most of the known facts; many non-menstruating animals have shorter cycles than women, including some non-menstruating primates; and many animals have more limited years of fertility than women. There is in any case little evidence that progesterone levels fall faster in women than in other animals at the end of the cycle. Whilst it is possible to induce menstruation in the cycle in some primates by ovariectomy (van Wagenen and Aberle, 1931; Cleveland, 1941), it is not possible in other cycling animals. Corner's suggestion is likely to be nearer the truth because it takes account of a fundamental difference between the methods of implantation found in different species. However, his view that the blood vessels open into the tissues in preparation for bleeding into the intervillous spaces, and the suggestion that it is the direct presence of the embryo which prevents the bleeding, could not take account of the later finding that menstruation can be prevented by the administration of extracts of the corpus luteum or exogenous progesterone (P.E. Smith and Engle, 1932; Corner, 1935). From this we now know that the blastocyst prevents menstruation indirectly by maintaining the corpus luteum and that opening of the vessels is the result of degenerative processes in the stroma. The problem is why the fall in progesterone at the end of the cycle causes bleeding in women and not in other animals. Recently, it has been suggested that this is due to a fundamental difference between the species in the processes taking place in the endometrium during the earlier part of the oestrous or menstrual cycle (Finn, 1986, 1987). It was noted earlier that during this time the tissues of the endometrium are prepared for implantation by cell proliferation and differentiation. However, complete differentiation of the stroma into the decidua does not take place in non-menstruating animals in the absence of a blastocyst and decidualization is not, therefore, part of the infertile cycle. In women, on the other hand, the blood vessels and stromal cells differentiate, even in the absence of a blastocyst, at the end of every cycle (Bartelmez, 1933).

All species require progesterone for stromal differentiation, and if the level of the hormone drops (as it does at the end of the menstrual cycle) then the decidualized tissue breaks down. It has been known for a considerable time that removal of the ovaries from pregnant animals causes degeneration of the placenta (Deanesly, 1972, 1973). Similarly, removal of the source of progesterone from animals in which a decidual reaction has been induced artificially causes decidual breakdown.

The cellular and vascular changes in the decidualized endometrium of mice following a fall in progesterone levels has been studied in detail to determine whether the changes can provide a clue to menstruation (Finn and Pope, 1984). Ovariectomized mice were prepared with oestrogen and progesterone and oil injected into the uterine lumen. No further hormone was given and animals were killed at intervals and their uteri examined histologically. The first change was a massive influx of leucocytes into the stroma. This is

the first observable change in the uterus of primates before menstruation (Novak and Te Linde, 1924). Leucocyte infiltration was followed by congestion of blood vessels with swollen erythrocytes, and then breakdown of the walls and extravasation of red blood corpuscles into the tissues. At the same time the decidual cells underwent typical degenerative changes and eventually a mass of blood and tissue debris was extruded into the lumen. In about 50% of cases a sanguinous discharge was clearly visible in the vagina, but the bleeding into the tissues was less than in menstruation. Nevertheless, the early invasion by leucocytes (Novak and Te Linde, 1924) and the extravasation of blood is reasonable evidence for the hypothesis of menstruation discussed above.

Further evidence comes from three non-primate animals which menstruate, the elephant shrew (*Elephantulus myurus jamesoni*) (van der Horst and Gilman, 1941) and the bats; *Glossophaga soricina* (Hamlett, 1934; Rasweiler, 1979) and *Molossus ater* (Rasweiler, 1991). The elephant shrew is a member of the family *Macroscelididae* and is closely related to primates (some authorities classify the *Macroscedididae* with the primates). It has a very interesting cellular reaction in the endometrial stroma during the late luteal phase of the cycle. A small area of each uterine horn forms a swollen polyp which is well vascularized and oedematous and contains large stromal cells with big nuclei which resemble decidual cells. At the end of the cycle the polyp breaks down and bleeding occurs from it. This naturally occurring phenomenon is very similar to the breakdown of the artificial deciduoma in mice. In the bat, *Molossus ater*, decidualization has been demonstrated to occur spontaneously (Rasweiler, 1991), whilst in *Glossophaga soricina* Hamlett (1934) found that the endometrium thickens greatly in preparation for implantation and then in the absence of a fertile egg the hypertrophied endometrium disintegrates and sloughs off with marked extravasation of leucocytes and blood cells. Further information on this species is needed.

The main non-human animal in which menstruation has been studied is probably the rhesus monkey (Hisaw, 1935), an Old World primate which menstruates as part of the cycle. These animals have a very unusual uterine response to the implanting blastocyst with the formation of a plaque of epithelial cells and very little if any decidual cell transformation of the stromal cells. However, the endometrium does undergo the early stages of the decidual reaction with intense development of the blood vessels (Phelps, 1946) and oedema of the stroma. These occur at the end of the cycle and are hormone dependent. Furthermore, there are changes during the cycle in the occurrence of the granulated K cells. In view of this it seems likely that transformation of stromal fibroblasts into decidual cells may not be the essential factor in menstruation but rather that the changes in the endometrial blood vessels and maybe in the extracellular matrix which precede implantation, or possibly factors originating in the granulated cells, are of greater importance.

It has been suggested that the presence of spiral arteries is the crucial factor in menstruation (Reynolds, 1947; S.T. Shaw and Roche, 1980), although this has been challenged on the basis that New World primates, which do not have spiral arteries, do menstruate. Furthermore, with a corrosion vascular casting/scanning electron microscope method, spiral arteries have been demonstrated in the rat endometrium (Rogers and Gannon, 1981), suggesting that too much significance should not be placed on the tortuosity of the arteries. This may simply be a reflection of growth of vessels relative to other stromal tissue, and not represent a fundamental difference in the vasculature of the two species.

3.6 EVOLUTION OF THE ENDOMETRIAL RESPONSE TO IMPLANTATION

From the brief introductory discussion of the various methods of implantation found in different species and the more detailed description of implantation changes, it is apparent that there is a gradually increasing complexity in the cellular response of the uterus to the blastocyst, culminating in the complex vascular and cellular changes of the decidual cell reaction. It is interesting to enquire what has caused this evolution and what advantage it has meant for the animal.

The evolution from the simplest forms of viviparity to deep embedding of the ovum in the wall of the uterus, whilst presumably conferring advantages on the species, will have presented considerable problems to the defence mechanisms of the animal. Very early in evolution animals had developed methods to combat and prevent the ingress into the tissues of foreign organisms (viruses, bacteria and parasites) and to counteract the trauma inflicted by them or by other means. Two interrelated mechanisms are involved – the inflammatory reaction and the immune response. Inflammation is the first to be encountered and is phylogenetically the more primitive response. Ryan and Majno (1977) describe it as an all-purpose defence system. Unless the animal could negate or overcome the inflammatory response of the endometrium internal development of embryos could not have progressed to deep implantation of the blastocyst in the uterine stroma.

3.6.1 INFLAMMATION AND DECIDUALIZATION

Many workers over the years have noted the similarity of the uterine response to an implanting blastocyst to that of inflammation, especially the similarity of the decidual reaction to the inflammatory granulation tissue reaction (Turner, 1873; Creighton, 1878). Fothergill (1899) suggested that the decidual cell had been evolved as a protection against the injurious invasion of the uterine wall by the fetal elements of the placenta, and Wade and Watson (1908) described the decidual cells as being similar to 'young fibroblasts such as are seen in the process of repair'. Loeb's classic discovery that trauma of the endometrium will stimulate a decidual reaction added credence to this view (Loeb, 1907). Several workers have reiterated this idea (Huggett and Hammond, 1952; Finn and Keen, 1963; Finn, 1986).

The fact that myofibroblasts in granulation tissue have gap junctions (Gabbiani *et al.*, 1971), similar to decidual cells, has also been noted (Finn, 1986). Psychoyos's (1960) finding that the endometrial blood vessels were more permeable in areas of the uterus in which blastocysts were implanting followed similar findings for tissues in which inflammation is taking place. Increased blood flow and vascular permeability are early signs of inflammation. Apart from the decidual cells, some of the other cells which are found in the endometrium at the time of decidualization are thought to be derived from the bone marrow, indicating a kinship between inflammation and the immune response. De *et al.* (1993) have shown expression of interleukin 1, interleukin 6 and tumour necrosis factor α in the mouse uterus during the peri-implantation period. These are normally associated with the inflammatory reaction.

Many of the mediators of the decidual response discussed earlier are involved in inflammation, for example histamine and prostaglandins, and at least one of the proteins produced by the decidua is similar to an acute-phase protein of inflammation.

The incidence of polymorphonuclear leucocytes is interesting. During the early stages of implantation in the mouse and rat or during the early stages of the oil decidual cell reaction there is an increase in the incidence of leucocytes in the stroma (Finn and Pope, 1991) preceded by leucocyte adhesion to the

endothelium (Tawia and Rogers, 1992). Once the decidual reaction is developed, however, polymorphs are rare. However, if the supply of progesterone is removed they appear in large numbers (Deanesly, 1972, 1973; Staples *et al.*, 1983; Finn and Pope, 1986), which of course happens in menstruation (Novak and Te Linde, 1924). Under these circumstances the course of events in the uterus follows fairly closely the acute inflammation response. It is suggested (Clemens *et al.*, 1977) that progesterone has an effect on the inflammatory reaction by altering cell motility and thus inhibiting ingress of leucocytes. In this way an acute inflammatory response to a foreign body is converted into a mechanism for protection of the embryo and possibly the uterus (Finn, 1986). In women this has evolved to the stage where the whole reaction proceeds without the presence of the blastocyst but in anticipation of it.

3.6.2 IMMUNE RESPONSE AND DECIDUALIZATION

Having converted the inflammatory response to the blastocyst into a protective rather than a destructive mechanism, there is still the problem of the immune response. The embryo and associated membranes will carry antigens foreign to the mother and it would be expected that the mother's immune mechanisms would mount an attack on them. The reason this does not happen has been the subject of intensive investigation (see Carter, 1984, for review). The main relevant point in the present context is the extent to which the cellular reaction at implantation is involved in preventing immunological rejection. Several workers (Tekelioglu-Uysal *et al.*, 1975; S.C. Bell, 1983b) have put forward the hypothesis that the decidual cell reaction protects the embryo from any immune reaction mounted by the mother.

Various lines of evidence point to such a immunological role. Some studies have shown variations in the infiltrating cells (bone marrow origin) around the time of implantation (Bernard *et al.*, 1981; Parr and Parr, 1986), and some cells in the mouse uterus have been shown to be immunosuppressive (Hunt *et al.*, 1984; Daya *et al.*, 1985; Clark and Chaouat, 1989). Extract of decidual tissue from rats suppresses primary antibody response *in vitro* (Kouttab *et al.*, 1976), and supernatants from short-lived cultures of mouse decidual cells have immunoregulatory activity (Badet *et al.*, 1983). Recently, D.A. Clark *et al.* (1990) have demonstrated an immunosuppressive molecule related to transforming growth factor β in decidua and various cytokines including interleukins, and colony-stimulating factors have been implicated (see D.A. Clark, 1993, for review). Type 1 interferon has also been demonstrated in the decidua (Yamamoto *et al.*, 1992).

The presence of immunoglobulins in metrial gland cells is well documented (D. Bulmer and Peel, 1977; Mitchell *et al.*, 1980, 1981; Matthews *et al.*, 1985), and their probable bone marrow origin was discussed earlier. It has been suggested that they are natural killer-like cells (E.L. Parr *et al.*, 1987, 1990; Ginsburg *et al.*, 1989; Linnemeyer and Pollack, 1991), which would indicate a role in the immunological response, as would the finding that they produce the cytokine interleukin 1 (Croy *et al.*, 1991).

3.6.3 OTHER POSSIBLE FUNCTIONS OF THE DECIDUA

If the decidua has evolved in response to the need to overcome problems associated with entry of the blastocyst into the stroma of the uterus then its primary function would be associated with this need for protection. The presence of tight junctions and immune competent cells would certainly point in this direction.

However, other functions have been suggested, and there is no reason to rule out the possibility that, having evolved in response to a protective need, other functions

have appeared later. Prolactin-like proteins synthesized by the decidua may have a role in maintaining the corpus luteum or in preparing the mammary gland for lactation, or they may be important in the control of water transport across fetal membranes.

The presence of large amounts of glycogen in decidua led some workers to suggest a nutrient role. Possibly the decidua acts as a reservoir of carbohydrate (glucose) for the developing embryo. Small molecules could easily pass through the gap junctions between decidual cells and into the blood vessels supplying the embryo or pass directly to the trophoblast.

DeFeo (1967) suggested that one of the functions of the decidua may be to ensure separation of developing placentae, especially of their blood vessels. If this were correct one would expect decidualization to be associated with polytocous species. This is clearly not the case. Sows give birth to litters containing many young but do not decidualize, whereas women usually have only one baby at a time but the uterine stroma undergoes considerable decidualization. Of course this does not mean that when decidualization occurs in polytocous species it does not have this desirable effect but, as discussed earlier, spacing of the blastocysts along the length of the uterus has occurred well before decidualization takes place, so it is unlikely that decidualization has evolved in response to the need for separation of the embryos.

It is difficult to explain several of the main features of decidual tissue with any of the suggested functions. Why do decidual cells have two nuclei which are mostly polyploid? What is the function of alkaline phosphatase? At present we do not have the answers to these and many other questions about this fascinating tissue.

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REFERENCES

Abrahamsohn, P., Lunkvist, O. and Nilsson, O. (1983) Ultrastructure of the endometrial blood vessels during implantation of the rat blastocyst. *Cell and Tissue Research*, **229**, 269–280.

Acker, G., Braquet, P. and Mencia-Huerta, J.M. (1989) Role of platelet-activating factor (PAF) in the initiation of the decidual reaction in the rat. *Journal of Reproduction and Fertility*, **85**, 623–629.

Adams, K.L., Bazer, F.W. and Roberts, R.M. (1981) Progesterone-induced secretion of a retinol-binding protein in the pig uterus. *Journal of Reproduction and Fertility*, **62**, 39–47.

Aitken, R.J. (1974) Delayed implantation in the roe deer (*Capreolus capreolus*). *Journal of Reproduction and Fertility*, **39**, 225–233.

Aitken, R.J. (1976) Uterine secretion of fructose in the roe deer. *Journal of Reproduction and Fertility*, **46**, 439–440.

Aitken, R.J. (1977a) The culture of mouse blastocysts in the presence of uterine flushings collected during normal pregnancy, delayed implantation and prooestrus. *Journal of Embryology and Experimental Morphology*, **41**, 295–330.

Aitken, R.J. (1977b) Changes in the protein content of mouse uterine flushings during normal pregnancy and delayed implantation and after ovariectomy and oestradiol administration. *Journal of Reproduction and Fertility*, **50**, 29–36.

Alberto-Rincon, M.C., Zorn, T.M. and Abrahamsohn, P.A. (1989) Diameter increase of collagen fibrils of the mouse endometrium during decidualization. *American Journal of Anatomy*, **186**, 417–429.

Aldeen, K.A.M. (1970) The influence of oestrogen and progesterone on the distribution of alkaline phosphatase in the mouse uterine endometrium. *Journal of Endocrinology*, **46**, 405–406.

Alden, R.H. (1947) Implantation of the rat egg. II. Alteration in osmophilic epithelial lipids of the rat uterus under normal and experimental conditions. *Anatomical Record*, **97**, 1–13.

Allen, E. and Doisy, E.A. (1923) An ovarian hormone: preliminary report on its localization, extraction and partial purification and action in

test animals. *Journal of the American Medical Association*, **81**, 819–821.

Allen, E., Smith, G.M. and Gardner, W.U. (1937) Accentuation of the growth effect of theelin on genital tissues of the ovariectomized mouse by arrest of mitosis with colchicine. *American Journal of Anatomy*, **61**, 321–341.

Allen, E., Thomas, T.B., Wilson, J.G. and Hession, D.A. (1943) Differential growth in the ovaries and genital tract near the time of ovulation in rats treated with colchicine. *American Journal of Anatomy*, **71**, 291–337.

Allen, W.M. (1931) Cyclical alterations of the endometrium of the rat during the normal cycle, pseudopregnancy and pregnancy. *Anatomical Record*, **48**, 65–84.

Allen, W.M. (1937) Some effects of oestrin and progestin in the rabbit. *Cold Spring Harbor Symposia on Quantitative Biology*, **5**, 66.

Allen, W.M. and Corner, G.W. (1929) Physiology of the corpus luteum. III. Normal growth and implantation of embryos after very early ablation of the ovaries under the influence of extracts of the corpus luteum. *American Journal of Physiology*, **88**, 340–346.

Allen, W.M. and Wintersteiner, O. (1934) Crystalline progestin. *Science*, New York, **80**, 190–191.

Allen, W.R., Hamilton, D.W. and Moor, R.M. (1973) Origin of equine endometrial cups. II. Invasion of the endometrium by the trophoblast. *Anatomical Record*, **177**, 485–502.

Alloiteau, J.J. and Psychoyos, A. (1966) Can the ovum of rats lose its zona pellucida in two ways? *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris*, **262**, 1561–1564.

Amoroso, E.C. (1952) Placentation, in *Marshall's Physiology of Reproduction*, 3rd edn, (ed. A.S. Parkes), Longman, Green, London, pp. 127–311.

Amoroso, E.C. (1981) Viviparity, in *Cellular and Molecular Aspects of Implantation*, (eds S.R. Glasser and D.W. Bullock), Plenum Press, New York, pp. 3–25.

Anderson, T.L. (1989) Biomolecular markers for the window of uterine receptivity, in *Blastocyst Implantation*, (ed. A. Yoshinaga), Serono Symposia USA, pp. 219–224.

Anderson, T.L. and Hoffman, L.H. (1984) Alterations in epithelial glycocalyx of rabbit uteri during early pseudopregnancy and pregnancy and following ovariectomy. *American Journal of Anatomy*, **171**, 321–334.

Anderson, T.L., Olson, G.E. and Hoffman, L.H. (1986) Stage specific alterations in the apical membrane glycoproteins of endometrial epithelial cells related to implantation in rabbits. *Biology of Reproduction*, **34**, 701–720.

Andersson, C.B. and Gustafsson, T.O. (1979) Delayed implantation in lactating bank voles, *Clethrionomys glareolus*. *Journal of Reproduction and Fertility*, **57**, 349–352.

Angle, M.J., Jones, M.A., Pinckard, R.N., McManus, L.M. and Harper, M.J.K. (1985) Platelet-activating factor (PAF) in the rabbit uterus during early pregnancy. *Biology of Reproduction*, **32**, Suppl. 143.

Ansell, J.D., Barlow, P.W. and McLaren, A. (1974) Binucleate and polyploid cells in the decidua of the mouse. *Journal of Embryology and Experimental Morphology*, **31**, 223–227.

Anteby, S.O., Bauminger, S., Zor, U. and Lindner, H.R. (1975) Prostaglandin synthesis in decidual tissue of the rat uterus. *Prostaglandins*, **10**, 991–999.

Aplin, J.D. (1991) Glycans as biochemical markers of human endometrial secretory differentiation. *Journal of Reproduction and Fertility*, **92**, 525–541.

Aplin, J.D., Charlton, A.K. and Ayad, S. (1988) An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and the first trimester of pregnancy. *Cell and Tissue Research*, **253**, 231–240.

Archer, G.T. (1959) The release of histamine from the mast cells of the rat. *Australian Journal of Experimental Biology and Medical Science*, **37**, 383–389.

Armant, D.R., Kaplin, H.A. and Lennarz, W.J. (1986) Fibronectin and laminin promote *in vitro* attachment and outgrowth of mouse blastocysts. *Developmental Biology*, **116**, 519–523.

Arzac, J.P. and Blanchet, E. (1948) Alkaline phosphatase and glycogen in human endometrium. *Journal of Clinical Endocrinology*, **8**, 315–324.

Asplund, J. and Holmgren, H.J. (1974) On the occurrence of the metachromatically granulated cells in the mucus membrane of the human uterus. *Acta Anatomica*, **3**, 312–320.

Astwood, E.B. (1938) A six hour assay for the quantitative determination of estrogen. *Endocrinology*, **23**, 25.

Atkinson, W.B. and Elftman, H. (1947) Mobilization of alkaline phosphatase in the uterus of the mouse by estrogen. *Endocrinology*, **40**, 30–36.

Atkinson, W.B. and Engle, E.T. (1947) Studies on

endometrial alkaline phosphatase during human menstrual cycle and in hormone treated monkey. *Endocrinology*, **40**, 327–33.

Bacsich, P. and Wyburn, G.M. (1940) Cyclic variations in the vascular architecture of the uterus of the guinea pig. *Transactions of the Royal Society of Edinburgh*, **60**, 79–86.

Bacsich, P. and Wyburn, G.M. (1941) Hormonal analysis of the cyclic variations in the vascular architecture of the uterus of the guinea pig. *Transactions of the Royal Society of Edinburgh*, **60**, 465–474.

Badet, M.T., Bell, S.C. and Billington, W.D. (1983) Immunoregulatory activity of supernatants from short-term cultures of mouse decidual tissue. *Journal of Reproduction and Fertility*, **68**, 351–358.

Baker, B.L. (1948) Histochemical variations in the metrial gland of the rat during pregnancy and lactation. *Proceedings of the Society of Experimental Biology and Medicine*, **68**, 492–496.

Baker, D.J., Nagy, F. and Nieder, G.L. (1992) Localization of c-*fos*-like proteins in the mouse endometrium during the peri-implantation period. *Biology of Reproduction*, **47**, 492–501.

Banik, U.K. and Ketchell, M.M. (1964) Inability of histamine to induce deciduomata in pregnant and pseudopregnant rats. *Journal of Reproduction and Fertility*, **7**, 259–261.

Barbiarz, B.S. and Hathaway, H.J. (1988) Hormonal control of the expression of antibody-defined lactosaminoglycans in the mouse uterus. *Biology of Reproduction*, **39**, 699–706.

Bartelmez, G.W. (1933) Histological studies on menstruating mucous membrane of human uterus. *Contributions to Embryology*, **24**, 141–186.

Bartocci, A., Pollard, J.W. and Stanley, E.R. (1986) Regulation of colony stimulating factors during pregnancy. *Journal of Experimental Medicine*, **164**, 956–961.

Basuray, R. and Gibori, G. (1980) Luteotrophic action of decidual tissue in the pregnant rat. *Biology of Reproduction*, **23**, 507–512.

Beard, J. (1897) The Span of Gestation in the Cause of Birth. *Jena*.

Beato, M., Lederer, B., Boqui, E. and Sandritter, W. (1968) Effect of estrogens and gestagens on the initiation of DNA synthesis in the genital tract of ovariectomized mice. *Experimental Cell Research*, **52**, 173–179.

Beier, H.M. (1968) Uteroglobin: A hormone-sensitive endometrial protein involved in blastocyst development. *Biochemica et Biophysica Acta*, **1609**, 289–291.

Bell, S.C. (1979a) Immunochemical identity of 'decidualization-associated protein' and $\alpha_2$acute-phase macroglobulin in the pregnant rat. *Journal of Reproduction and Immunology*, **1**, 193–206.

Bell, S.C. (1979b) Synthesis of 'decidualization-associated protein' in tissues of the rat uterus and placenta during pregnancy. *Journal of Reproduction and Fertility*, **56**, 255–262.

Bell, S.C. (1983a) Decidualization: Regional differentiation and associated function, in *Oxford Reviews of Reproductive Biology*, Vol. 5 (ed. C.A. Finn), Clarendon Press Oxford, pp. 220–271.

Bell, S.C. (1983b) Decidualization and associated cell types: Implications for the role of the placental bed in the materno-fetal immunological relationship. *Journal of Reproductive Immunology*, **5**, 185–194.

Bell, S.C. (1985) Comparative aspects of decidualization in rodents and human: cell types, secreted products and associated function, in *Implantation of the human embryo*, (eds R.G. Edwards, J.M. Purdy and P.C. Steptoe), Academic Press, London, pp. 71–122.

Bell, S.C. (1986) Secretory endometrial and decidual proteins: studies and clinical significance of a maternally derived group of pregnancy-associated serum proteins. *Human Reproduction*, **1**, (3), 129–143.

Bell, S.C., Fazleabas, T.T. and Verhage, G. (1989) Comparative aspects of secretory proteins of the endometrium and decidua in the human and non-human primates, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA. pp. 151–162.

Bell, S.C., Patel, S.R., Jackson, J.A. and Waites, G.T. (1988) Major secretory protein of human decidualized endometrium in pregnancy is an insulin-like growth factor-binding protein. *Journal of Endocrinology*, **118**, 317–328.

Bell, W.B. and Hicks, P. (1909) Observations on the physiology of the female genital organs. III. The correlation of the uterus and ovaries. *British Medical Journal*, **i**, 655–658.

Bensley, C.M. (1951) Cyclic fluctuations in the rate of epithelial mitosis in the endometrium of the rhesus monkey. *Contributions to Embryology*, Publications of the Carnegie Institution, 34, 87–98.

Bernard, O., Scheid, M., Ripoche, M.A. and Bennett, D. (1978) Immunological studies of mouse decidual cells. 1. Membrane markers of

decidual cells in the days after implantation. *Journal of Experimental Medicine*, **148**, 580–591.

Bernard, O., Rachman, F. and Bennett, D. (1981) Immunoglobulins in the mouse uterus before implantation. *Journal of Reproduction and Fertility*, **63**, 237–240.

Bertalanffy, F.D. and Lau, C. (1963) Mitotic rates, renewal times and cytodynamics of the female genital tract epithelia in the rat. *Acta Anatomica*, **54**, 39–81.

Bevilacqua, E.M. and Abrahamsohn, P.A. (1989) Trophoblast invasion during implantation of the mouse embryo. *Archivos de Biologia y Medicina Experimentales Santiago*, **22**, 107–118.

Bhatt, H., Brunet, L.J. and Stewart, C.L. (1991) Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *Proceedings of the National Academy of Sciences of the USA*, **88**, 11408–11412.

Biggers, J.D., Finn, C.A. and McLaren, A. (1962) Long-term reproductive performance of female mice. II. Variation of litter size with parity. *Journal of Reproduction and Fertility*, **3**, 313–330.

Bindon, B.M. (1969) Blood flow in the reproductive organs of the mouse after hypophysectomy, after gonadotrophin treatment, during the oestrous cycle and during early pregnancy. *Journal of Endocrinology*, **44**, 523–536.

Bindon, B.M. (1971) Role of progesterone in implantation in the sheep. *Journal of Reproduction and Fertility*, **24**, 146.

Bischof, P. (1981) Pregnancy-associated plasma protein-A: an inhibitor of the complement system. *Placenta*, **2**, 29–34.

Bischof, P., Geinoz, A., Herrmann, W.L. and Sizonenko, P.C. (1984) Pregnancy-associated plasma protein A (PAPP-A) specifically inhibits the third component of human complement (C3). *Placenta*, **5**, 1–8.

Bitton-Casimiri, V., Brun, J.L. and Psychoyos, A. (1976) Uptake and incorporation of [^{3}H] uridine by normal or diapausing rat blastocysts after various periods of culture. *Journal of Reproduction and Fertility*, **46**, 447–448.

Blandau, R.J. (1949) Observations on implantation of the guinea pig ovum. *Anatomical Record*, **103**, 19–47.

Blankenship, T.N. and Given, R.L. (1992) Penetration of the uterine epithelial basement membrane during blastocyst implantation in the mouse. *Anatomical Record*, **233**, 196–204.

Bonnet, R. (1882) Die Uterinmilch und ihre Bedeutung fur die Frucht, Stuttgart.

Boshier, D.P. (1969) A histological and histochemical examination of implantation and early placentome formation in sheep. *Journal of Reproduction and Fertility*, **19**, 51–61.

Boshier, D.P. (1970) The pontamine sky blue reaction in pregnant sheep uteri. *Journal of Reproduction and Fertility*, **22**, 595–596.

Boshier, D.P., Holloway, H. and Millener, N.M. (1981) Triacylglycerols in the rat uterine epithelium during oestrous cycle and early pregnancy. *Journal of Reproduction and Fertility*, **62**, 441–446.

Bouda, J. (1969) Arterioles and capillaries of human decidua during the first trimester of pregnancy. *Obstetrique et Gynecologie*, **33**, 182–188.

Boving, B. (1954) Blastocyst-uterine relationships. *Cold Spring Harbor Symposia on Quantitative Biology*, **19**, 9–25.

Boyd, J.D. and Hamilton, W.J. (1952) Cleavage, early development and implantation of the egg, in *Marshall's Physiology of Reproduction*, 3rd edn, Vol II (ed. A.S. Parkes), Longman, Green, London, pp. 1–126.

Brandon, J.M. and Bibby, M.C. (1979) A study of changes in uterine mast cells during early pregnancy in the rat. *Biology of Reproduction*, **20**, 977–980.

Brandon, J.M. and Wallis, R.M. (1977) Effect of mepyramine, a histamine H_1-, and burimamide, a histamine H_2- receptor antagonist, on ovum implantation in the rat. *Journal of Reproduction and Fertility*, **50**, 251–254.

Bray, J. and Craggs, R. (1979) The characterisation of $F_c$receptors in the metrial gland of the pregnant rat uterus. *Journal of Reproductive Immunology*, **1**, 109–115.

Bray, J., Stewart, I. and Craggs, R. (1978) The demonstration of cells bearing $F_c$receptors in the metrial gland of pregnant rat uterus. *Cell Tissue Research*, **192**, 89–96.

Brigstock, D.R. (1988) Heparin-binding growth factors from the porcine uterus. *PhD Thesis*, University of Cambridge.

Brigstock, D.R. (1991) Growth factors in the uterus: steroidal regulation and biological actions. *Balliere's Clinical Endocrinology and Metabolism*, **5**, 791–808.

Brigstock, D.R., Heap, R.B. and Brown, K.D. (1989) Polypeptide growth factors in uterine tissues and secretions. *Journal of Reproduction and Fertility*, **85**, 747–758.

Brinsfield, T.H. and Hawk, H.W. (1974) Ultrastructure of sheep endometrial stromal

cells after ovariectomy and hormone treatment. *Biology of Reproduction*, **10**, 98–102.

Brouha, L. (1928) Production of placentoma in rats injected with anterior hypophysial fluid. *Proceedings of the Society of Experimental Biology and Medicine*, **25**, 488–489.

Brown, J.J.G. and Papaioannou, V.E. (1992) Distribution of hyaluronan in the mouse endometrium during the peri-implantation period of pregnancy. *Differentiation*, **5**, 61–68.

Bryce, T.H. and Teacher, J.H. (1908) Contributions to the study of the early development and imbedding of the human ovum. Glasgow.

Bulmer, D. (1968) Further studies on the granulated metrial gland cells of the pregnant rat. *Journal of Anatomy*, **103**, 479–487.

Bulmer, D. and Peel, S. (1977) The demonstration of immunoglobulin in the metrial gland cells of the rat placenta. *Journal of Reproduction and Fertility*, **49**, 143–145.

Bulmer, J.N. and Sunderland, C.A. (1983) Bone marrow origin of endometrial granulocytes in the early placental bed. *Journal of Reproductive Immunology*, **5**, 383–387.

Bulmer, J.N., Stewart, I.J., Mitchell, B.S. and Peel, S. (1980) Endometrial granulocytes in the human uterus. *Journal of Anatomy*, **131**, 754.

Bulmer, J.N., Stewart, I. and Peel, S. (1983) Endometrial granulocytes of the pregnant hamster. *Journal of Anatomy*, **136**, 329–337.

Bulmer, J.N., Hollings, D. and Ritson, A. (1987) Immunocytochemical evidence that endometrial stromal granulocytes are granulated lymphocytes. *Journal of Pathology*, **153**, 281–288.

Burgos, M.H. and Wislocki, G.B. (1958) The cyclic changes in the mucosa of the guinea pig uterus, cervix and vagina and in the sexual skin investigated by electron microscopy. *Endocrinology*, **63**, 106–121.

Burin, J. and Sartor, P. (1965) Inhibition de la réaction déciduale par L'actinomycine D. *Comptes Rendus Hebdomadaires des Seances et Memoires de la Société de Biologie, Paris*, **159**, 141–144.

Butenandt, A. and Westphal, U. (1934) Zur Isolierung und Charakterisierung des Corpus Luteum Hormone. *Bericht der Deutschen Chemishchen Gesellschaft*, **67**, 1440–1442.

Camus, M., Lejeune, B. and Leroy, F. (1979) Induction of implantation in the rat by intraparametrial injection of actinomycin D. *Biology of Reproduction*, **20**, 1115–1118.

Canivenc, R. and Bonnin-Laffargue, M. (1963) Inventory of problems raised by the delayed implantation in the European badger (*Meles meles L*), in *Delayed Implantation*, (ed. A.C. Enders), University Press, Chicago, pp. 115–129.

Canivenc, R., Laffargue, M. and Mayer, G. (1956) Nidations retardées chez la ratte castrée et injecté de progesterone: influence du moment de la castration sur la chronologie de l'ovo-implantation. *Comptes rendus hebdomadaires des séances et memoires de la Société de Biologie Paris*, **150**, 2208–2212.

Cardell, R.R., Hisaw, F.L. and Dawson, A.B. (1969) The fine structure of granular cells in the uterine endometrium of the rhesus monkey (*Macaca mulatta*) with a discussion of the possible function of these cells in relaxin secretion. *American Journal of Anatomy*, **124**, 307–340.

Carter, J. (1984) The maternal immunological response during pregnancy, in *Oxford Reviews of Reproductive Biology*, Vol. 6 (ed. J. Clarke), Oxford University Press, Oxford, pp. 47–128.

Carter, J. and McLaren, A. (1975) The effect of oestrogen and progesterone on the incorporation of tritiated thymidine in mouse uteri *in vitro*. *Journal of Reproduction and Fertility*, **42**, 439–445.

Castracane, V.D.D., Saksena, S.K. and Shaikh, A.A. (1974) Effect of IUD's, prostaglandins and indomethacin on decidual cell reaction in the rat. *Prostaglandins*, **6**, 397–404.

Cejic, S.S. and Kennedy, T.G. (1991) Effects of piriprost (U-60, 257B) and leukotrienes on alkaline phosphatase activity of rat endometrial stromal cells *in vitro*. *Prostaglandins*, **42**, 163–179.

Chavez, D.J. (1986) Cell surface of mouse blastocysts at the trophectoderm-uterine interface during the adhesive stage of implantation. *American Journal of Anatomy*, **176**, 153–158.

Chavez, D.J. and Anderson, T.L. (1985) The glycocalyx of the mouse uterine luminal epithelium during estrus, early pregnancy, the peri-implantation period and delayed implantation. *Biology of Reproduction*, **32**, 1135–1142.

Cheng, S.V.Y. and Pollard, J.W. (1986) C-*ras* and ornithine decarboxylase are induced by oestradiol-17β in the mouse uterine luminal epithelium independently of the proliferative status of the cell. *FEBS Letters*, **196**, 309–314.

Christofferson, R.H. and Nilsson, B.O. (1988) Morphology of the endometrial microvasculature during early placentation in the rat. *Cell and Tissue Research*, **253**, 209–220.

Clark, B.F. (1971) The effects of oestrogen and progesterone on uterine cell division and epithelial morphology in spayed, adrenalectomized rats. *Journal of Endocrinology*, **50**, 527–528.

Clark, D.A. (1993) Cytokines, decidua and early pregnancy, in *Oxford Reviews of Reproductive Biology*, Vol. 15 (ed. S. Milligan), Clarendon Press Oxford. (in press)

Clark, D.A. and Chaouat, G. (1989) Determinants of embryo survival in the peri- and post-implantation period, in *Blastocyst Implantation* (ed. K. Yoshinaga) Serona Symposia, Adams, Boston, pp171–178.

Clark, D.A., Flanders, K.C., Banwatt, D., Millar-Brook, W., Manuel, J., Stedronska-Clark, J. and Rowley, B. (1990) Murine pregnancy decidua produces a unique immunosuppressive molecule related to transforming growth factor β-2. *Journal of Immunology*, **144**, 3008–3014.

Clark, M.J. (1968) Termination of embryonic diapause in the red kangaroo, *Megeleia rufa*, by injection of progesterone or oestrogen. *Journal of Reproduction and Fertility*, **15**, 347–355.

Clemens, L.W., Contopoulos, A.N., Ortiz, S., Stites, D.P. and Siiteri, P.K. (1977) Inhibition by progesterone of leucocyte migration *in vivo* and *in vitro*, in *Abstracts of the 59th Annual meeting of Endocrine Society*, **237**, 175.

Clemetson, C.A.B., Moshfeighi, M.M. and Mallikarjuneswara, V.R. (1970) The surface charge on the five-day rat blastocyst, in *Biology of the Blastocyst*, (ed. R.J. Blandau), University of Chicago Press, pp. 193–205.

Cleveland, R. (1941) Observations on hormone withdrawal bleeding in castrated macaques. *Endocrinology*, **29**, 343–348.

Cochrane, R.L. and Meyer, R.K. (1957) Delayed nidation in the rat induced by progesterone. *Proceedings of the Society of Experimental Biology and Medicine*, **96**, 155–159.

Collawn, S.S., Rankin, J.C., Ledford, B.E. and Baggett, B. (1981) Ornithine decarboxylase activity in the artificially stimulated decidual cell reaction in the mouse uterus. *Biology of Reproduction*, **24**, 528–533.

Concannon, P., Pilbeam, T. and Travis, H. (1980) Advanced implantation in mink (*Mustela vison*) treated with medroxyprogesterone acetate during early embryonic diapause. *Journal of Reproduction and Fertility*, **58**, 1–6.

Corner, G.W. (1921) Cyclic changes in the ovaries and uterus of the sow and their relation to the mechanisms of implantation. *Contribution to Embryology (Publications of the Carnegie Institution)*, **13**, 119–121.

Corner, G.W. (1923) Oestrus, ovulation and menstruation. *Physiological Review*, **3**, 457–482.

Corner, G.W. (1928) Physiology of the corpus luteum. 1. The effect of very early ablation of the CL upon embryos and uterus. *American Journal of Physiology*, **86**, 74–81.

Corner, G.W. (1935) Influence of the ovarian hormones, oestrin and progestin, upon the menstrual cycle of the monkey. *American Journal of Physiology*, **113**, 238–250.

Corner, G.W. and Warren, S.L. (1919) Influence of the ovaries upon the production of artificial deciduomata: confirmatory studies, *Anatomical Record*, **16**, 168–169.

Courrier, R. and Kehl, R. (1930) Le déciduome expérimental chez la lapine gestante. *Comptes Rendus Hebdomadaires des Séances et Memoires de la Société de Biologie, Paris*, **104**, 1180–1181.

Creighton, C. (1878) On the formation of the placenta in the guinea pig. *Journal of Anatomy and Physiology*, **XIII**, 534–590.

Croy, B.A., Guilbert, L.J., Browne, M.A., Gough, N.M., Stinchcomb, D.T., Reed, N. and Wegmann, T.G. (1991) Characterization of cytokine production by the metrial gland and granulated metrial gland cells. *Journal of Reproductive Immunology*, **19**, 149–166.

Cullingford, T.E. and Pollard, J.W. (1988) RU486 completely inhibits the action of progesterone on cell proliferation in the mouse uterus. *Journal of Reproduction and Fertility*, **83**, 909–914.

Dallenbach-Hellweg, G. (1967) Endometrial granulocytes and implantation. *Excerpta Medica*, International Congress Series 133, 411–418.

Daniel, J.C. (1970) Coincidence of embryonic growth and uterine protein in the ferret. *Journal of Embryology and Experimental Morphology*, **24**, 305–312.

Daniel, S.A.J. and Kennedy, T.G. (1987) Prostaglandin E_2 enhances uterine stromal cell alkaline phosphatase activity *in vitro*. *Prostaglandins*, **33**, 241–252.

Daron, G.H. (1936) The arterial pattern of the tunica mucosa of the uterus in macacus rhesus. *American Journal of Anatomy*, **58**, 349–419.

Davies, J. and Hoffman, L.H. (1973) Studies on the progestational endometrium of the rabbit. 1. Light microscopy day 0–13 of gonadotrophin-induced pseudopregnancy. *American Journal of Anatomy*, **137**, 423–445.

Daya, S., Clark, D.A., Devlin, C. and Jarrell, J.

(1985) Preliminary characterization of two types of suppressor cells in the human uterus. *Fertility and Sterility*, **44**, 778–785.

De, M., Sanford, T.R. and Wood, G.W. (1993) Expression of interleukin 1, interleukin 6 and tumour necrosis factor α in mouse uterus during the peri-implantation period of pregnancy. *Journal of Reproduction and Fertility*, **98**, 83–89.

Deanesly, R. (1960) Implantation and early pregnancy in ovariectomized guinea pigs. *Journal of Reproduction and Fertility*, **1**, 242–248.

Deanesly, R. (1972) Retarded embryonic development and pregnancy termination in ovariectomized guinea pigs: progesterone deficiency and decidual collapse. *Journal of Reproduction and Fertility*, **28**, 241–247.

Deanesly, R. (1973) Termination of early pregnancy in rats after ovariectomy is due to immediate collapse of the progesterone-dependent decidua. *Journal of Reproduction and Fertility*, **35**, 183–186.

DeFeo, V.J. (1962) Comparative effectiveness of several methods for the production of deciduomata in the rat. *Anatomical Record*, **142**, 226.

DeFeo, V.J. (1967) Decidualization, in *Cellular Biology of the Uterus*, (ed. R.M. Wynn), Appleton-Century-Crofts, New York, pp. 191–291.

Dent, J. (1973) Ultrastructural changes in the intercotyledonary placenta of the goat during early pregnancy. *Journal of Anatomy*, **114**, 245–259.

Dey, S.K., Johnson, D.C. and Santos, J.G. (1979) Is histamine production by the blastocyst required for implantation in the rabbit? *Biology of Reproduction*, **21**, 1169–1173.

DiAugustine, R.P., Petrusz, P., Bell, G.I., Brown, C.F., Korach, K.S., McLachlan, J.A. and Teng, C.T. (1988) Influence of estrogens on mouse uterine epidermal growth factor precursor protein and messenger ribonucleic acid. *Endocrinology*, **122**, 2355–2362.

Dickson, A.D. and Bulmer, D. (1961) Observations of the origin of metrial gland cells in the rat placenta. *Journal of Anatomy*, **95**, 262–273.

Dickson, W.M., Bosc, M.J. and Locatelli, A. (1969) Effect of estrogen and progesterone on uterine blood flow of castrate sows. *American Journal of Physiology*, **271**, 1431–1434.

Duncan, D.A., Mazur, M.T. and Younger, J.B. (1988) Glycoconjugate distribution in normal human endometrium. A lectin histochemical study. *International Journal of Gynecology and Pathology*, **7**, 236–248.

Durham, W.C. (1961) Progestational steroid requirements for inducing and maintaining decidua in women. *Fertility and Sterility*, **12**, 45–54.

Duval, M. (1891) La placenta des rongeurs. *Journal et l'Anatomie et de la Physiologie, Paris*, **27**, 24.

Edwards, C. and Milligan, S.R. (1987) Uterine blood flow during the development and regression of the decidual reaction in ovariectomized steroid treated mice. *Journal of Reproduction and Fertility*, **81**, 525–532.

Eichner, E., Goler, G.G., Reed, J. and Gordon, M.B. (1951) The experimental production and prolonged maintenance of decidua in the non pregnant woman. *American Journal of Obstetrics and Gynecology*, **61**, 253–264.

Einer-Jenson, N. (1977) Endometrial blood flow in rats during early pregnancy. *Journal of Reproduction and Fertility*, **49**, 377–379.

Emrys-Roberts, E. (1909) The embedding of the embryo guinea pig in the uterine wall and its nutrition at that stage of development. *Journal of Anatomy and Physiology*, **44**, 192–206.

Enders, A.C. and Nelson, D.M. (1973) Pinocytotic activity of the uterus of the rat. *American Journal of Anatomy*, **138**, 277–299.

Enders, A.C. and Schlafke, S. (1967) A morphological analysis of the early implantation stages in the rat. *American Journal of Anatomy*, **120**, 185–226.

Enders, A.C. and Schlafke, S. (1969) Cytological aspects of trophoblast – uterine interaction in early pregnancy. *American Journal of Anatomy*, **125**, 1–30.

Enders, A.C. and Schlafke, S. (1971) Penetration of the uterine epithelium during implantation in the rabbit. *American Journal of Anatomy*, **132**, 219–240.

Enders, A.C. and Schlafke, S. (1972) Implantation in the ferret: Epithelial penetration. *American Journal of Anatomy*, **133**, 291–299.

Enders, A.C. and Schlafke, S. (1974) Surface coats of the mouse blastocyst and uterus during the preimplantation period. *Anatomical Record*, **180**, 31–45.

Enders, A.C., Schlafke, S. and Welsh, A.O. (1980) Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in the rat. *American Journal of Anatomy*, **159**, 59–72.

Enders, A.C., Schlafke, S., Hubbard, N.E. and Mead, R.A. (1986) Morphological changes in the blastocyst of the western spotted skunk during

activation from delayed implantation. *Biology of Reproduction*, **34**, 423–437.

Epifanova, O.I. (1966) Mitotic cycles in estrogen-treated mice: A radioautographic study. *Experimental Cell Research*, **42**, 562–577.

Evans, C.A. and Kennedy, T.G. (1978) The importance of prostaglandin synthesis for the initiation of blastocyst implantation in the hamster. *Journal of Reproduction and Fertility*, **54**, 255–261.

Everett, J. (1962) The influence of oestradiol and progesterone on the endometrium of the guinea pig *in vitro*. *Journal of Endocrinology*, **24**, 491–496.

Everett, J. (1963) Action of oestrogens and progesterone on uterine epithelial mitosis in organ culture. *Nature*, **198**, 896–898.

Fagg, B., Martin, L., Rogers, L., Clark, B. and Quarmby, E. (1979) A simple method for removing the luminal epithelium of the mouse uterus for biochemical studies. *Journal of Reproduction and Fertility*, **57**, 335–339.

Farach, M.C., Tang, J.P., Decker, G.L. and Carson, D.D. (1987) Heparin/heparan sulfate is involved in attachment and spreading of mouse embryos *in vitro*. *Developmental Biology*, **123**, 401–410.

Fawcett, D.W. (1950) The development of mouse ova under the capsule of the kidney. *Anatomical Record*, **108**, 71–84.

Fawcett, D.A., Wislocki, G.B. and Waldo, C.M. (1947) The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *American Journal of Anatomy*, **81**, 13–443.

Fernandez-Noval, A. and Leroy, F. (1978) Induction of implantation in the mouse by intrauterine injection of adenosine monophosphate. *Journal of Reproduction and Fertility*, **54**, 7–8.

Finn, C.A. (1965) Oestrogen and the decidual cell reaction of implantation in mice. *Journal of Endocrinology*, **32**, 223–229.

Finn, C.A. (1966a) Endocrine control of endometrial sensitivity during the induction of the decidual cell reaction in the mouse. *Journal of Endocrinology*, **36**, 239–248.

Finn, C.A. (1966b) The initiation of the decidual cell reaction in the uterus of the aged mouse. *Journal of Reproduction and Fertility*, **11**, 423–428.

Finn, C.A. (1968) Increase in length of the uterus at the time of implantation in the mouse. *Journal of Reproductive Fertility*, **17**, 69–74.

Finn, C.A. (1970) The ageing uterus and its influence on reproductive capacity. *Journal of Reproduction and Fertility*. Suppl. 12, 31–38.

Finn, C.A. (1971) The biology of decidual cells. *Advances in Reproductive Physiology*, **5**, 1–26.

Finn, C.A. (1974) The induction of implantation in mice by actinomycin D. *Journal of Endocrinology*, **60**, 199–200.

Finn, C.A. (1980) Species variation in implantation. *Progress in Reproductive Biology*, **7**, 253–261.

Finn, C.A. (1983) Implantation of ova – assessment of the value of laboratory animals as models for the study of implantation in women, in *Oxford Reviews of Reproductive Biology*, Vol. 5, Clarendon Press Oxford, pp. 272–289.

Finn, C.A. (1986) Implantation, menstruation and inflammation. *Biological Reviews*, **61**, 313–328.

Finn, C.A. (1987) Why do women and some other primates menstruate? *Perspectives in Biology and Medicine*, **30**, 566–574.

Finn, C.A. (1989) Species variation, location and attachment of blastocysts, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 47–54.

Finn, C.A. and Bredl, J.C.S. (1973) Studies on the development of the implantation reaction in the mouse uterus: Influence of actinomycin D. *Journal of Reproduction and Fertility*, **34**, 247–253.

Finn, C.A. and Bredl, J.C.S. (1975) The development of embryos in the uteri of mice treated with actinomycin D before implantation. *Journal of Embryology and Experimental Morphology*, **34**, 429–434.

Finn, C.A. and Bredl, J.C.S. (1977) Autoradiographic study of the effect of actinomycin D on decidual differentiation of stromal cells in the mouse uterus. *Journal of Reproduction and Fertility*, **50**, 109–111.

Finn, C.A. and Hinchliffe, J.R. (1964) Reaction of the mouse uterus during implantation and deciduoma formation as demonstrated by changes in the distribution of alkaline phosphatase. *Journal of Reproduction and Fertility*, **8**, 331–338.

Finn, C.A. and Hinchliffe, J.R. (1965) Histological and histochemical analysis of the formation of implantation chambers in the mouse uterus. *Journal of Reproduction and Fertility*, **9**, 301–309.

Finn, C.A. and Keen, P.M. (1962a) Studies on deciduomata formation in the rat. *Journal of Reproduction and Fertility*, **4**, 215–216.

Finn, C.A. and Keen, P.M. (1962b) Failure of histamine to induce deciduoma in the rat. *Nature*, **194**, 602–603.

Finn, C.A. and Keen, P.M. (1962c) Influence of systemic antihistamines on formation of deciduoma. *Journal of Endocrinology*, **24**, 381–382.

Finn, C.A. and Keen, P.M. (1963) The induction of deciduomata in the rat. *Journal of Embryology and Experimental Morphology*, **11**, 673–682.

Finn, C.A. and Lawn, A.M. (1967) Specialized junctions between decidual cells in the uterus of the pregnant mouse. *Journal of Ultrastructure Research*, **20**, 321–327.

Finn, C.A. and Lawn, A.M. (1968) Transfer of cellular material between the uterine epithelium and trophoblast during the early stages of implantation. *Journal of Reproduction and Fertility*, **15**, 333–336.

Finn, C.A. and McLaren, A. (1967) A study of the early stages of implantation in mice. *Journal of Reproduction and Fertility*, **13**, 259–267.

Finn, C.A. and Martin, L. (1967) Patterns of cell division in the mouse uterus during early pregnancy. *Journal of Endocrinology*, **39**, 593–597.

Finn, C.A. and Martin, L. (1969a) Hormone secretion during early pregnancy in the mouse. *Journal of Endocrinology*, **45**, 57–65.

Finn, C.A. and Martin, L. (1969b) The cellular response of the uterus of the aged mouse to oestrogen and progesterone. *Journal of Reproduction and Fertility*, **20**, 545–547.

Finn, C.A. and Martin, L. (1970) The role of the oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *Journal of Endocrinology*, **47**, 431–438.

Finn, C.A. and Martin, L. (1971) Endocrine control of the proliferation and secretion of uterine glands in the mouse. *Acta Endocrinologica*, Suppl. 155, 139.

Finn, C.A. and Martin, L. (1972a) Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biology of Reproduction*, **7**, 82–86.

Finn, C.A. and Martin, L. (1972b) Temporary interruption of the morphogenesis of deciduomata in the mouse uterus by actinomycin D. *Journal of Reproduction and Fertility*, **31**, 353–358.

Finn, C.A. and Martin, L. (1973) Endocrine control of gland proliferation in the mouse uterus. *Biology of Reproduction*, **8**, 585–588.

Finn, C.A. and Martin, L. (1976) Hormonal control of the secretion of the endometrial glands in the mouse. *Journal of Endocrinology*, **71**, 273–274.

Finn, C.A. and Pollard, R.M. (1973) The influence of the oestrogen secreted before oestrus on the timing of endometrial sensitivity and insensitivity during implantation. *Journal of Endocrinology*, **56**, 619–620.

Finn, C.A. and Pope, M. (1984) Vascular and cellular changes in the decidualized endometrium of the ovariectomized mouse following cessation of hormone treatment: a possible model for menstruation. *Journal of Endocrinology*, **100**, 295–300.

Finn, C.A. and Pope, M. (1986) Control of leucocyte infiltration into the decidualized mouse uterus. *Journal of Endocrinology*, **110**, 93–96.

Finn, C.A. and Pope, M.D. (1989) The positioning of blastocysts and the decidual cell reaction in the mouse. *Journal of Reproduction and Fertility*, Abstract Series 3, p. 53.

Finn, C.A. and Pope, M.D. (1991) Infiltration of neutrophil polymorphonuclear leucocytes into the endometrial stroma at the time of implantation of ova and the initiation of the oil decidual cell reaction in mice. *Journal of Reproduction and Fertility*, **91**, 356–369.

Finn, C.A. and Publicover, M. (1981) Cell proliferation and cell death in the endometrium, in *Cellular and Molecular Aspects of Implantation*, (eds S.W. Glasser and D.W. Bullock), Plenum Publishing Corporation, New York, pp. 181–195.

Finn, C.A., Pope, M.D. and Milligan, S.R. (1991) Timing of the window of uterine sensitivity in mice. *Journal of Reproduction and Fertility*, Abs. Ser. **4**, 565–571.

Ford, S.P. and Christenson, R.K. (1979) Blood flow to uteri of sows during the estrus cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. *Biology of Reproduction*, **21**, 617–624.

Ford, S.P., Weber, L.J. and Stormshak, F. (1977) Role of oestradiol-17β and progesterone in regulating constriction of ovine uterine arteries. *Biology of Reproduction*, **17**, 480–483.

Fortier, M.A., Boulet, A.P. and Lambert, R.D. (1989) Regulation of adenylate cyclase activity and stimulation response in relation to endometrial receptivity in the rabbit. *Journal of Reproduction and Fertility*, **85**, 443–450.

Fothergill, W.E. (1899) The function of decidual cells. *Edinburgh Medical Journal*, **V**, 265–273.

Fox, H. (1985) The Endometrium, in *Muir's Textbook of Pathology*, (ed. J.R. Anderson), Edward Arnold, London, pp. 246–248.

Fraenkel, L. and Cohn, F. (1901) Experimentelle untersuchung uber den einfluss des corpus

luteum und die insertion des eies. *Anatomisher Anzeiger*, **20**, 294–300.

Frank, R.T. (1911) The function of the ovary. *Surgery, Gynecology and Obstetrics*, **13**, 36–53.

Frommel, R. (1883) Beitrag zur Frage der Wachstumsrichtung der Placenta. *Zentralblatt fur die gesamte Gynakologie und Geburtschilfe (Berlin)*, **36**, 490.

Gabbiani, G., Ryan, G.B. and Majno, G. (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia*, **27**, 549–550.

Galassi, L. (1967) Reutilization of maternal nuclear material by embryonic and trophoblastic cells in the rat for the synthesis of deoxyribonucleic acid. *Journal of Histochemistry and Cytochemistry*, **15**, 573–579.

Galassi, L. (1968) Autoradiographic study of the decidual cell reaction in the rat. *Developmental Biology*, **17**, 75–84.

Gambel, P., Rossant, J., Hunziker, R.D. and Wegmann, T.G. (1985) Decidual cells in murine pregnancy and pseudopregnancy: origin and natural killer activity. *Transplant Proceedings*, **17**, 75–84.

Gardner, R.L. (1975) Analysis of determination and differentiation in the early mammalian embryo using intra- and inter-specific chimeras, in *The Developmental Biology of Reproduction: 33rd Symposium of The Society for Developmental Biology*, (eds C.L. Markert and J. Papaconstantinou), Academic Press, New York, pp. 207–236.

Garris, D.R. (1984) Uterine blood flow, pH and pCO_2 during nidation in the guinea pig: ovarian regulation. *Endocrinology*, **114**, 1219–1224.

Garris, D.R. and Whitehead, D.A. (1981) Uterine blood flow and timing of blastocyst implantation in the guinea pig. *American Journal of Physiology*, **241**, E142–E145.

Gasbarrini, A. (1911) Su una forma particolare di reazione della mucosa uterina in seguito ad alcuni traumi (Placentazione artificiale di Leo Loeb). *Internationale Monatschrift Anatomie und Physiologie*, **28**, 259–265.

Gibbons, A.F.E. and Chang, M.C. (1972) Number of mast cells in the rat uterus with special reference to its relation to hormonal treatment and decidual response. *Biology of Reproduction*, **6**, 193–203.

Gibori, G., Rothchild, I., Pepe, G.J., Morishige, W.K. and Lam, P. (1974) Luteotropic action of decidual tissue in the rat. *Endocrinology*, **95**, 1113–1118.

Gilman, J. (1941) Profound vascular changes induced in the uterus of the castrated rabbit by combinations of estradiol benzoate and progesterone. *Endocrinology*, **29**, 336–342.

Ginsburg, H., Coleman, R., Davidson, S., Khoury, C. and Mor, R. (1989) Lymphokine-activated killer (LAK) cells are identical to the uterine granulated metrial gland (GMG) cells. *Transplant Proceedings*, **21**, 186–189.

Given, R.L. and Enders, A.C. (1980) Mouse uterine glands during the pre-implantation period: fine structure. *American Journal of Anatomy*, **157**, 169–179.

Given, R.L. and Enders, A.C. (1981) Mouse uterine glands during the peri-implantation period. II. Autoradiographic studies. *Anatomical Record*, **199**, 109–127.

Glasser, S.R. (1972) The uterine environment in implantation and decidualization, in *Excerpta Medica Foundation*, (eds H.A. Balin and S.R. Glasser), Amsterdam, pp. 776–883.

Glasser, S.R. and Clark, J.H. (1975) A determinant role for progesterone in the development of uterine sensitivity to decidualization and ovoimplantation, in *Developmental Biology of Reproduction: 33rd Symposium of The Society for Developmental Biology*, (eds C.l. Markert and J. Papaconstantinou), Academic Press, New York, pp. 311–345.

Glasser, S.R. and Julian, J. (1986) Intermediate filament protein as a marker of uterine stromal cell decidualization. *Biology of Reproduction*, **35**, 463–474.

Golander, A., Hurley, T., Barrett, J., Hizi, A. and Handwerger, S. (1978) Prolactin synthesis in the amniotic fluid. *Science*, **202**, 311–313.

Gompel, C. (1964) Structure fine des mitochondries de la cellulé glandulaire endomètriale humaine au cours du cycle menstruel. *Journal of Microscopy*, **3**, 427–436.

Good, R.G. and Moyer, D.L. (1968) Estrogen-progesterone relationships in the development of secretory endometrium. *Fertility and Sterility*, **19**, 37–49.

Goodger, A.M. and Rogers, D.A.W. (1993) Uterine endothelial cell proliferation before and after implantation in the rat. *Journal of Reproduction and Fertility*, **94** (in press).

Grant, E. (1978) *A Source Book of Medieval Science*, Harvard University Press, Cambridge, Massachusetts.

Green, E. (1979) Early pregnancy in guinea pigs. *PhD Thesis*, Liverpool University.

Greenwald, G.S. and Everett, N.B. (1959) The incorporation of S^{35} methionine by the uterus and ova of mice. *Anatomical Record*, **134**, 171–184.

Greiss, F.C. and Anderson, S.G. (1969) Uterine vascular changes during the ovarian cycle. *American Journal of Obstetrics and Gynecology*, **103**, 629–640.

Grinnell, F., Head, J.R. and Hoffpauir, J. (1982) Fibronectin and cell shape *in vivo*: Studies on the endometrium during pregnancy. *Journal of Cell Biology*, **94**, 597–606.

Grosser, O. (1910) The development of the egg membranes and the placenta; menstruation, in *Manual of Human Embryology*, (eds F. Keibel and F.P. Mall), J.B. Lippincott, Philadelphia and London.

Guillomot, M. and Guay, P. (1982) Ultrastructural features of the cell surfaces of uterine and trophoblastic epithelia during embryo attachment in the cow. *Anatomical Record*, **204**, 315–322.

Guillomot, M., Flechon, J.E. and Winterberger-Torres, S. (1981) Conceptus attachment in the ewe: an ultrastructural study. *Placenta*, **2**, 169–182.

Guillomot, M., Flechon, J.E. and Winterberger-Torres, S. (1982) Cytochemical studies of uterine and trophoblastic surface coats during blastocyst attachment in the ewe. *Journal of Reproduction and Fertility*, **65**, 1–8.

Gulyas, B.J. and Daniel, J.C. (1969) Incorporation of labelled nucleic acid and protein precursors by diapausing and non-diapausing blastocysts. *Biology of Reproduction*, **1**, 11–20.

Gwatkin, R.B.L. (1966) Amino acid requirements for attachment and outgrowth of the mouse blastocyst *in vitro*. *Journal of Cellular Physiology*, **68**, 335–344.

Hall, K. (1968) Disappearance of histochemically-demonstrable adneosine triphosphatase and of pas-reactive basement membranes in blood vessels in the decidua during ovum implantation in mice. *Journal of Endocrinology*, **41**, 53–59.

Hall, K. (1969) Uterine mitosis, alkaline phosphatase and adenosine triphosphatase during development and regression of deciduomata in pseudopregnant mice. *Journal of Endocrinology*, **44**, 91–100.

Ham, K.N., Hurley, J.V., Lopata, A. and Ryan, G.B. (1970) A combined isotopic and electron microscopic study of the response of the rat uterus to exogenous oestradiol. *Journal of Endocrinology*, **46**, 71–81.

Hamilton, P.A. (1973) The spacing of the blastocysts in mice – a growth response of the uterus at the time of implantation. *Journal of Anatomy*, **114**, 309–310.

Hamlett, G.W. (1934) Uterine bleeding in a bat *Glossophaga soricina*. *Anatomical Record*, **60**, 9–17.

Hammond, J. (1935) The changes in the reproductive organs of the rabbit during pregnancy. *Transactions of Dynamics of Growth*, **10**, 10–93.

Hamper, L.H. (1955) The granular endometrial stroma cells. A new cell type. *Journal of Pathology and Bacteriology*, **69**, 358–359.

Hansson, A. (1947) The physiology of reproduction in mink (*Mustella vison, Screb*) with special reference to delayed implantation. *Acta Zoologica*, **28**, 1–136.

Harper, M.J.K., Prostkoff, B.T. and Reeve, R.J. (1966) Implantation and embryonic development in the ovariectomised hamster. *Acta Endocrinologica*, (Copenhagen) **52**, 465–470.

Hartman, C.G. (1932) Studies in the reproduction of the monkey Maracus (Pithecus) rhesus with special reference to menstruation and pregnancy. *Contributions to Embryology, Carnegie Institution*, **23**, 1–161.

Hatanaka, K., Kitamura, Y., Maeyama, K., Watanabe, T. and Matsumoto, K. (1982) Deciduoma formation in uterus of genetically mast cell deficient w/w^v mice. *Biology of Reproduction*, **27**, 25–28.

Heald, P.J. (1979) Changes in ornithine decarboxylase during early implantation in the rat. *Biology of Reproduction*, **20**, 1195–1199.

Heald, P.J. and O'Hare, A. (1973) Changes in rat uterine nuclear RNA during early pregnancy. *Biochimica et Biophysica Acta*, **324**, 86–92.

Heald, P.J., O'Grady, J.E. and Moffatt, G.E. (1970) The uptake of [^{3}H] uridine into the nucleic acids of rat uterus during early pregnancy. *Biochemical Journal*, **117**, 65–71.

Heald, P.J., O'Grady, J.E. and Moffatt, G.E. (1972) The incorporation of ^{3}H-uridine into nuclear RNA in the uterus of the rat during early pregnancy. *Biochimica et Biophysica Acta*, **281**, 347–352.

Heap, R.B., Fleet, I.R., Finn, C., Ellis, S.T., Yang, C.B., Whyte, A. and Brigstock, D.R. (1988) Maternal reactions affecting early embryonogenesis and implantation. *Journal of Reproduction and Fertility*, Suppl. 36, 83–97.

Heape, W. (1900) The 'sexual season' of mammals and the relation of the 'proestrum' to menstruation. *Quarterly Journal of Microscopical Science*, **44**, 1–70.

Hedlund, K., Nilsson, O., Reinius, S. and Aman, G. (1972) Attachment reaction of the uterine luminal epithelium at implantation: light and electron microscopy of the hamster, guinea-pig, rabbit and mink. *Journal of Reproduction and Fertility*, **29**, 131–132.

Henning, C. (1872) Studien uber den Bau der menschlichen Placenta etc. Leipzig.

Hertig, A.T. and Rock, J. (1941) Two human ova of the pre-villous stage, having an ovulation age of about 11 and 12 days respectively. *Contributions to Embryology*, **29**, 127–156.

Hertig, A.T. and Rock, J. (1945) Two human ova of the pre-villous stage, having a developmental age of about seven and nine days respectively. *Contributions to Embryology*, **31**, 65–84.

Heuser, C.H. (1940) The chimpanzee ovum in the early stages of implantation (about 10½ days). *Journal of Morphology*, **66**, 155–173.

Heuser, C.H. and Streeter, G.L. (1941) Development of the macaque embryo. *Contributions to Embryology*, **29**, 15–55.

Hewitt, K., Beer, A.E. and Grinnell, F. (1979) Disappearance of anionic sites from the surface of the rat endometrial epithelium at the time of implantation. *Biology of Reproduction*, **21**, 691–707.

Heyner, S. (1981) Immunogenetic approaches to the study of the mammalian egg and early embryo, in *Oxford Reviews of Reproductive Biology*, Vol. 3, (ed. C.A. Finn), Clarendon Press, Oxford, pp. 95–126.

Hial, V., DeMello, M.C.F., Horakova, Z. and Beavan, M.A. (1977) Antiproliferative activity of anti-inflammatory drugs in the two mammalian cell culture lines. *Journal of Pharmacology and Experimental Therapeutics*, **202**, 446–454.

Hinds, L.A. (1989) Pregnancy deferred: an unusual role for prolactin in a marsupial, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 201–208.

Hisaw, F.L. (1935) The physiology of menstruation in Macacus rhesus monkeys. *American Journal of Obstetrics and Gynecology*, **29**, 638.

Hitschmann, F. and Adler, L. (1908) Der Bar der Uterusschleimhaut der geschlechtsreifen Weibes mit besonderer Berucksichtigung der Menstruation. *Monatschrift fur Geburtshilfe und Gynakologie*, **27**, 1–82.

Hoffman, L.H. (1978) Antifertility effect of indomethacin during early pregnancy in the rabbit. *Biology of Reproduction*, **18**, 148–153.

Hoffman, L.H. and Hoos, P.C. (1984) Morphology of vascular leakage at rabbit implantation sites. *Anatomical Record*, **208**, 75A.

Hoffman, L.H. and Strong, G.B. (1976) Prostaglandin-induced decidual development in rabbits. *Anatomical Record*, **184**, 430.

Hoffman, L.H., Strong, G.B., Davenport, G.R. and Frolich, J.C. (1977) Deciduogenic effect of prostaglandins in the pseudopregnant rabbit. *Journal of Reproduction and Fertility*, **50**, 231–237.

Holmes, P.V. and Bergstrom, (1975) Induction of blastocyst implantation in mice by cyclic AMP. *Journal of Reproduction and Fertility*, **43**, 329–332.

Holmes, P.V. and Dickson, A.D. (1973) Estrogen-induced surface coat and enzyme changes in the implanting mouse blastocyst. *Journal of Embryology and Experimental Morphology*, **29**, 639–645.

Hong, L., Mulholland, J., Chinsky, J.M., Knudson, T.B., Kellems, R.E. and Glasser, S.R. (1991) Developmental expression of adenosine deaminase during decidualization in the rat uterus. *Biology of Reproduction*, **44**, 83–93.

Hooker, C.W. (1945) A criterion of luteal activity in the mouse. *Anatomical Record*, **93**, 333–347.

Hoos, P.C. and Hoffman, L.H. (1980) Temporal aspects of rabbit uterine vascular and decidual responses to blastocyst stimulation. *Biological Reproduction*, **23**, 453–459.

van der Horst, C.J. and Gilman, J. (1941) The menstrual cycle in elephantulus. *South African Journal of Medical Science*, **6**, 27–47.

Hoyes, A.D. (1972) The endometrial glands of the pregnant sheep: an ultrastructural study. *Journal of Anatomy*, **111**, 55–67.

Huet-Hudson, Y.M., Chakraborty, C., Dey, S.K., Suzuki, Y., Andrews, G.K. and Dey, S.K. (1990) Estrogen regulates the synthesis of epidermal growth factor in mouse uterine epithelial cells. *Molecular Endocrinology*, **4**, 510–523.

Huggett, A.St G. and Hammond, J. (1952) Physiology of the placenta, in (ed. A.S. Parkes), *Marshall's Physiology of Reproduction*, Vol. 2, Longman, Green, London, pp. 312–397.

Humphrey, K.W. and Martin, L. (1968) Attempted induction of deciduomata in mice with mast-cell, capillary permeability and tissue inflammatory factors. *Journal of Endocrinology*, **42**, 129–141.

Hunt, J.S., Manning, L.S. and Wood, G.W. (1984) Macrophages in murine uterus are immunosuppressive. *Cell Immunology*, **85**, 499–510.

Hunter, W. (1777) *Anatomy of the Human Gravid Uterus*, Birmingham, UK.

Hunter, W. (1794) *An anatomical description of the human grạvid uterus and its contents*, J. Johnson, London.

Huxley, T.H. (1869) *Introduction to Classification*, London.

Jayatilak, P.F., Puryear, T.K., Herz, Z., Fazleabas, A. and Gibori, G. (1989) Expression of mRNAs and synthesis of proteins by rat antimesometrial and mesometrial decidua, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 145–150.

Jelinek, J. and Jelinkova, M. (1977) Rat endometrium enzymes in 4-day oestrous cycle and early pregnancy. *Acta Endocrinologica, (Copenhagen)*, **85**, 169–176.

Jenkinson, E.J. and Searle, R.F. (1977) Cell surface changes on the mouse blastocyst at implantation. *Experimental Cell Research*, **106**, 386–390.

Johnson. S.A., Morgan, G. and Wooding, F.B.A. (1988) Alterations in uterine epithelial tight junction structure during the oestrous cycle and implantation in the pig. *Journal of Reproduction and Fertility*, 83, 915–922.

Jollie, W.P. and Benscosme, S.A. (1965) Electron microscopic observations on primary decidua formation in the rat. *American Journal of Anatomy*, **116**, 217–236.

Jonsson, H.T., Rankin, J.C., Ledford, B.E. and Baggett, B. (1979) Uterine prostaglandin levels following stimulation of the decidual cell reaction: effects of indomethacin and tranylcypromine. *Prostaglandins*, **18**, 847–857.

Julian, J., Carson, D.D. and Glasser, S.R. (1992) Polarized rat uterine epithelium *in vitro*: responses to estrogen in defined medium. *Endocrinology*, **130**, 68–78.

Kaiser, I.H. (1948) Histological appearance of coiled arteries in endometrium of rhesus monkey, baboon, chimpanzee and gibbon. *Anatomical Record*, **99**, 199–225.

Kapur, S., Tamada, H., Dey, S.K. and Andrews, G.K. (1992) Expression of insulin-like growth factor-1 (IGF-1) and its receptor in the periimplantation mouse uterus, and cell-specific regulation of IGF-1 gene expression by estradiol and progesterone. *Biology of Reproduction*, **46**, 208–219.

Karkavelas, G., Kefalides, N.A., Amenta, P.S. and Martinez-Hermandez, A. (1988) Comparative ultrastructural localization of collagen types III, IV, VI and laminin in rat uterus and kidney. *Journal of Ultrastructure and Molecular Structure Research*, **100**, 137–155.

Kearns, M. and Lala, P.K. (1982) Bone marrow origin of decidual cell precursors in the pseudopregnant mouse uterus. *Journal of Experimental Medicine*, **155**, 1537–1554.

Kennedy, T.G. (1977) Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat. *Biology of Reproduction*, **16**, 286–291.

Kennedy, T.G. (1979) Prostaglandins and increased endometrial vascular permeability resulting from the application of an artificial stimulus to the uterus of the rat sensitized for the decidual cell reaction. *Biology of Reproduction*, **20**, 560–566.

Kennedy, T.G. (1983) Prostaglandin E_2, adenosine 3′:5′ cyclic monophosphate and changes in endometrial vascular permeability in rat uteri sensitized for decidual cell reaction. *Biology of Reproduction*, **29**, 1069–1076.

Kennedy, T.G. (1985) Evidence for the involvement of prostaglandins throughout the decidual cell reaction in the rat. *Biology of Reproduction*, **33**, 140–146.

Kennedy, T.G. and Doktorcik, P.E. (1988) Uterine decidualization in hypophysectomized – ovariectomized rats: effects of pituitary hormones. *Biology of Reproduction*, **39**, 318–328.

Kennedy, T.G. and Samecnik, J. (1978) The concentration of 6-keto-prostaglandin F_1 is markedly elevated at the site of blastocyst implantation in the rat. *Prostaglandins*, **16**, 599–605.

Kennedy, T.G., Martel, D. and Psychoyos, A. (1983) Endometrial prostaglandin E_2 binding: characterization in rats sensitized for the decidual cell reaction and changes during pseudopregnancy. *Biology of Reproduction*, **29**, 565–571.

Keys, J.L. and King, G.J. (1985) Changes in vascular permeability and endometrial structure during early gestation in the pig. *Biology of Reproduction*, **32**, Suppl. 1, pp. 63.

Keys, J.L. and King, G.J. (1988) Morphological evidence for increased uterine vascular permeability at the time of embryonic attachment in the pig. *Biology of Reproduction*, **39**, 473–487.

Kimber, S.J. and Lindenberg, S. (1990) Hormonal control of a carbohydrate epitope involved in implantation in mice. *Journal of Reproduction and Fertility*, **89**, 13–21.

Kirby, D.R.S. (1960) Development of mouse eggs beneath the kidney capsule. *Nature*, **187**, 707–708.

Kirby, D.R.S. (1963a) The development of mouse

blastocysts transplanted to the scrotal and cryptorchid testis. *Journal of Anatomy*, **97**, 119–130.

Kirby, D.R.S. (1963b) Development of the mouse blastocyst transplanted to the spleen. *Journal of Reproduction and Fertility*, **5**, 1–12.

Kirby, D.R.S., Potts, D.M. and Wilson, I.B. (1967) On the orientation of the implanting blastocyst. *Journal of Embryology and Experimental Morphology*, **17**, 527–532.

Kirchner, C. (1975) Vorbereitungen aut die implantatin beim kaninchen. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **67**, 142–145.

Kirkham, W.B. (1916) The prolonged gestation period in suckling mice. *Anatomical Record*, **11**, 31–40.

Kirkland, J.L., Murthy, L. and Stancel, G.M. (1992) Progesterone inhibits the estrogen-induced expression of c-fos messenger ribonucleic acid in the uterus. *Endocrinology*, **130**, 3223–3230.

Kirkwood, K.J. (1981) Immunoglobulin and complement receptor-bearing cells in cultures of mouse decidual tissue. *Journal of Reproduction and Fertility*, **62**, 345–352.

Kisalus, L.L., Herr, J.C. and Little, C.D. (1987) Immunolocalization of extracellular matrix proteins and collagen synthesis in first-trimester human decidua. *Anatomical Record*, **218**, 402–415.

Kleinfeld, R.G., Morrow, H.A. and DeFeo, V.J. (1976) Intercellular junctions between decidual cells in the growing deciduoma of the pseudopregnant rat uterus. *Biology of Reproduction*, **15**, 593–603.

Knoth, M. and Larsen, F.J. (1972) Ultrastructure of a human implantation site. *Acta Obstetrica et Gynecologica Scandinavica*, **51**, 593–603.

Knudson, T.B., Blackburn, M.R., Chinsky, J.M., Airhart, M.J. and Kellems, R.E. (1991) Ontogeny of adenosine deaminase in the mouse decidua and placenta: immunolocalization and embryo transfer studies. *Biology of Reproduction*, **44**, 171–184.

Kohorn, E.I. and Tchao, R. (1969) Conversion of proliferative endometrium to secretory epithelium by progesterone in organ culture. *Journal of Endocrinology*, **45**, 401–405.

Koj, A. (1974) Acute phase reactants. Their synthesis, turnover and biological significance, in *Structure and Function of Plasma Proteins*, Vol 1, (ed. A.C. Allison), Plenum Press, London and New York, pp. 73–132.

Kouttab, N.M., Fowler, A.K., Strickland, J.E. and Hellman, A. (1976) Suppression of *in vitro* lymphocyte stimulation in mice by uterine and placental extracts. *Journal of Immunology*, **117**, 1644–1650.

Kranz, K. (1914) Uber Reizwirkungen van Fremdkorpern aut die Uterusschleimhaut der Hunde. *Archiv fur Mikroskopische Anatomie und Entwicklungsmechanik*, **84**, 122.

Krehbiel, R.H. (1937) Cytological studies of the decidual reaction in the rat during early pregnancy and in the production of deciduomata. *Physiological Zoology*, **10**, 212–233.

Krehbiel, R.H. (1941) The effects of theelin on delayed implantation in the pregnant lactating rat. *Anatomical Record*, **81**, 381–392.

Krichesky, B. (1943) Vascular changes in the rabbit uterus and in intraocular endometrial transplants during pregnancy. *Anatomical Record*, **87**, 221–233.

Krishnan, R.S. and Daniel, J.C. (1967) 'Blastokinin': inducer and regulator of blastocyst development in the rabbit uterus. *Science*, **158**, 490–492.

Krueger, W.A. and Craig-Maibenco, H. (1972) DNA replication and cell division in the hamster uterus. *Anatomical Record*, **173**, 229–234.

Kubo, H., Spindle, A. and Pederson, R.A. (1981) Inhibition of mouse blastocyst attachment and outgrowth by protease inhibitors. *Journal of Experimental Zoology*, **216**, 445–451.

Kwun, J.K. and Emmens, V.W. (1974) Hormonal requirements for implantation and pregnancy in the ovariectomised rabbit. *Australian Journal of Biological Sciences*, **27**, 275–283.

Kyd, J.M. and Murdoch, R.N. (1992) Deciduogenic effects of mediators of the polyphosphatidylinositol pathway in pseudopregnant mice. *Experientia*, **48**, 741–745.

Ladinski, L.J. (1902) Deciduoma malignum – a clinical review. *American Journal of Obstetrics and Diseases of Women*, **XLV**, 465–509.

Lala, P.K. and Graham, C.H. (1990) Mechanisms of trophoblast invasiveness and their control: the role of proteases and protease inhibitors. *Cancer Metastasis Review*, **9**, 369–379.

Lampelo, S.A., Ricketts, A.P. and Bullock, D.W. (1985) Purification of rabbit endometrial plasma membranes from receptive and non-receptive uteri. *Journal of Reproduction and Fertility*, **75**, 475–484.

Langhans, T.H. (1890) Ueber glykogen in pathologischen neubildungen und den menschlichen eihauten. *Virchows Archiv fur Pathologische Anatomie und Physiologie*, **CXX**, 28–67.

Langston, W.C. and Robinson, B.L. (1935) Castration atrophy. A chronological study of uterine changes following ovariectomy in the albino rat. *Endocrinology*, **19**, 51–62.

Larkin, L.H. and Cardell, R.R. (1971) Differentiation of granulated metrial cells in the uterus of the pregnant rat: A electron microscopic study. *American Journal of Anatomy*, **132**, 241–258.

Larkin, L.H. and Flickinger, C.J. (1969) Ultrastructure of the metrial gland cell in the pregnant rat. *American Journal of Anatomy*, **126**, 337–354.

Larkin, L.H. and Schultz, R.L. (1968) Histochemical and autoradiographic studies of the formation of the metrial gland in the pregnant rat. *American Journal of Anatomy*, **122**, 607–620.

Larsen, J.F. (1961) Electron microscopy of the implantation site in the rabbit. *American Journal of Anatomy*, **109**, 319–334.

Larsen, J.F. (1970) Electron microscopy of nidation in the rabbit and observations on the human trophoblast invasion, in *Ovo-implantation, Human Gonadotropins and Prolactin*, (eds P.O. Hubinot, F. Leroy, C. Robyn and P. Leleux), Karger, Basel, pp. 38–51.

Lataste, F. (1887) Recherches de Zooetique sur les Mammiferes de l'ordre des Rongeurs. *Acta Societe Linnaeus, (Bordeaux)*, **40**, 202.

Lataste, F. (1891) Des variations de durée de la gestation chez les mammifères et des circonstances qui déterminent ces variations. Théorie de la gestation retardée. *Comptes Rendus Hebdomadaires des Seances et Memoires de la Societe de Biologie, Paris*, **43**, 21–31.

Lawn, A.M., Wilson, E.W. and Finn, C.A. (1971) The ultrastructure of human decidual and predecidual cells. *Journal of Reproduction and Fertility*, **26**, 85–90.

Lea, R.G., Flanders, K.C., Harley, C.B., Manuel, J., Banwatt, D. and Clark, D.A. (1992) Release of a transforming growth factor (TGF)-β 2-related suppressor factor from postimplantation murine decidual tissue can be correlated with the detection of a subpopulation of cells containing RNA for TGF-β2. *Journal of Immunology*, **148**, 778–787.

Ledford, B.E., Rankin, J.C., Frofle, V.L., Serra, M.J., Markwald, R.R. and Baggett, B. (1978) The decidual cell reaction in the muse uterus: DNA synthesis and autoradiographic analysis of responsive cells. *Biology of Reproduction*, **18**, 506–509.

Lee, A.E. and Dukelow, W.R. (1972) Synthesis of DNA and mitosis in rabbit uteri after oestrogen and progesterone injection, and during early pregnancy. *Journal of Reproduction and Fertility*, **31**, 473–476.

Lee, M.C. and Damjanov, I. (1985) Pregnancy-related changes in the human endometrium revealed by lectin histochemistry. *Histochemistry*, **82**, 275–280.

Lee, M.C., Wu, T.C., Wan, Y.J. and Damjanov, I. (1983) Pregnancy related changes in the mouse oviduct and uterus revealed by differential binding of fluoresceinated lectins. *Histochemistry*, **79**, 365–375.

Lejeune, B., van Hoeck, J. and Leroy, F. (1981) Transmitter role of the luminal epithelium in the induction of decidualization in rats. *Journal of Reproduction and Fertility*, **61**, 235–240.

Lejeune, B., Lecocq, R., Lamy, F. and Leroy, F. (1982) Changes in the pattern of endometrial protein synthesis during decidualization in the rat. *Journal of Reproduction and Fertility*, **66**, 519–523.

Leroy, F. and Galand, P. (1969) Radioautographic evaluation of mitotic parameters in the endometrium during the uterine sensitivity period in the pseudopregnant rat. *Fertility and Sterility*, **20**, 980–992.

Leroy, F., Galand, P. and Chretien, J. (1969) The mitogenic action of ovarian hormones on the uterine and vaginal epithelium during the oestrous cycle in the rat: a radioautographic study. *Journal of Endocrinology*, **45**, 441–447.

Leroy, F., Bogaert, C., van Hoeck, J. and Delcroix, C. (1974a) Cytophotometric and autoradiographic evaluation of cell kinetics in decidual growth in rats. *Journal of Reproduction and Fertility*, **38**, 441–449.

Leroy, F., Vansande, J., Shetgen, G. and Brasseur, D. (1974b) Cyclic AMP and the triggering of the decidual reaction. *Journal of Reproduction and Fertility*, **39**, 207–211.

Leroy, F., van Hoeck, J. and Bogaert, C. (1976) Hormonal control of pinocytosis in the uterine epithelium of the rat. *Journal of Reproduction and Fertility*, **47**, 59–62.

Lin, T.H., Venkat, R.M., Verner, G., Kirkland, J.L. and Stancel, G.M. (1988) Autoradiographic localization of epidermal growth factor receptors to all major uterine cell types. *Biology of Reproduction*, Vol. 38, 403–411.

Lindenberg, S. Hyttel, P. Lenz, S. and Holmes, P.V. (1986) Ultrastucture of the early human

implantation *in vitro*. *Human Reproduction*, **1**, 533–538.

Lingham, R.B., Stancel, G.M. and Loose-Mitchell, D.S. (1988) Estrogen regulation of epidermal growth factor receptor messenger ribonucleic acid. *Molecular Endocrinology*, **2**, 230–235.

Linnemeyer, P.A. and Pollack, S.B. (1991) Murine granulated metrial gland cells at uterine implantation sites are natural killer lineage cells. *Journal of Immunology*, **147**, 2530–2535.

Ljungkvist, I. (1971a) Attachment reaction of rat luminal epithelium. I. Gross and fine structure of the endometrium of the spayed virgin rat. *Acta Societatis Medicorum Upsaliensis*, **76**, 91–109.

Ljungkvist, I. (1971b) Attachment reaction of the rat uterine luminal epithelium. II. The effect of progesterone on the morphology of the uterine glands and the luminal epithelium of the spayed virgin rat. *Acta Societatis Medicorum Upsaliensis*, **76**, 110–126.

Ljungkvist, I. (1971c) Attachment of the rat uterine luminal epithelium. III. The effect of estradiol, estrone and oestriol on the morphology of the luminal epithelium of the spayed virgin rat. *Acta Societatis Medicorum Upsaliensis*, **76**, 139–157.

Ljungkvist, I. (1972) Attachment reaction of rat uterine luminal epithelium. IV. The cellular changes in the attachment reaction and its hormonal control. *Fertility and Sterility*, **23**, 847–865.

Lloyd, C.W. (1937) Effect of progesterone on cell division in the uterine epithelium. *Proceedings of the Society of Experimental Biology and Medicine*, **36**, 190–191.

Lobel, B.L., Tic, L. and Shelesnyak, M.C. (1965) Studies on the mechanisms of nidation. XVII. Histochemical analysis of decidualization in the rat. *Acta Endocrinologica, (Copenhagen)*, **50**, 537–539.

Loeb, L. (1907) Wounds of the pregnant uterus. *Proceedings of the Society of Experimental Biology and Medicine*, **4**, 93–94.

Loeb, L. (1908a) The experimental production of the maternal placenta and the formation of the corpus luteum. *Journal of American Medical Association*, **50**, 1897–1901.

Loeb, L. (1908b) The experimental production of the maternal part of the placenta in the rabbit. *Proceedings of the Society of Experimental Biology and Medicine*, **5**, 102–104.

Loeb, L. (1923) The effect of extirpation of the uterus on the life of the corpus luteum in the guinea pig. *Proceedings of the Society of Experimental Biology, New York*, **20**, 441.

Loeb, L., Sunteff, V. and Burns, E.L. (1938) Growth processes induced by estrogenic hormones in the uterus of the mouse. *American Journal of Cancer*, **34**, 413–427.

Long, J.A. and Evans, H.E. (1922) The oestrous cycle in the rat and its associated phenomena. *Memoirs of University of California*, **6**, 1–148.

Loose-Mitchell, D.A., Chiapetta, C. and Stancel, G.M. (1988) Estrogen regulation of c-fos messenger ribonucleic acid. *Molecular Endocrinology*, **2**, 946–51.

Lowenstein, W.R. and Penn, R.D. (1967) Intercellular communication and tissue growth. *Journal of Cell Biology*, **33**, 235–242.

Lunam, C. and Rogers, A.W. (1981) Pericytes in the stroma of the rat uterus. *Journal of Reproduction and Fertility*, **63**, 267–270.

Luxford, K.A. and Murphy, C.R. (1989) Cytoskeletal alterations in the microvilli of uterine epithelial cells during early pregnancy. *Acta Histochemistry*, **87**, 131–136.

Luxford, K.A. and Murphy, C.R. (1992) Changes in the apical microfilaments of rat uterine epithelial cells in response to estradiol and progesterone. *Anatomical Record*, **233**, 521–526.

McLaren, A. (1968) A study of blastocysts during delay and subsequent implantation in lactating mice. *Journal of Endocrinology*, **42**, 453–468.

McLaren, A. (1969) A note on the zona pellucida in mice. *Advances in Reproductive Physiology*, **4**, 207–210.

McLaren, A. (1970) The fate of the zona pellucida in mice. *Journal of Embryology and Experimental Morphology*, **23**, 1–19.

McLaren, A. and Biggers, J.D. (1958) Successful development and birth of mice cultivated *in vitro* as early embryos. *Nature*, **182**, 877–878.

McLaren, A. and Michie, D. (1956) Studies on the transfer of fertilized mouse eggs to uterine foster mothers. 1. Factors affecting the implantation and survival of native and transferred eggs. *Journal of Experimental Biology*, **33**, 394–416.

McLaren, A. and Michie, D. (1959) The spacing of implantations in the mouse uterus, in *Implantation of Ova*, (ed. P. Eckstein), *Memoirs of the Society of Endocrinology*, **6**, 65–74.

McPhail, M.K. (1934) The assay of progestin. *Journal of Physiology*, **83**, 145–156.

Manning, J.P., Meli, A. and Steintz, B.G. (1966) Alkaline phosphatase and β-glucuronidase activity in the rat uterus during early pregnancy. *Journal of Endocrinology*, **35**, 385–391.

Marcus, G.J. (1974a) Mitosis in the rat uterus

during the estrous cycle, early pregnancy, and early pseudopregnancy. *Biology of Reproduction*, **10**, 447–452.

Marcus, G.J. (1974b) Hormonal control of proliferation in the guinea-pig uterus. *Journal of Endocrinology*, **63**, 89–97.

Marcus, G.J. and Shelesnyak, M.C. (1967) Studies on the mechanism of nidation XXVI. Pro-oestrous oestrogen as a hormonal parameter of nidation. *Endocrinology*, **80**, 1038–1042.

Markee, J.E. (1929) Rhythmic variations in the vascularity of the uterus of the guinea pig during the oestrous cycle. *American Journal of Obstetrics and Gynecology*, **17**, 205–208.

Marshall, F.H.A. (1910) *The Physiology of Reproduction*, Longman, Green and Co., London.

Martel, D., Monier, M.-N., Psychoyos, A. and DeFeo, V.J. (1984) Estrogen and progesterone receptors in the endometrium, myometrium and metrial gland of the rat during the decidualization process. *Endocrinology*, **114**, 1627–1634.

Martel, D., Frydman, R., Glissant, M., Maggioni, C., Roche, D. and Psychoyos, A. (1987) Scanning electron microscopy of postovulatory human endometrium in spontaneous cycles and cycles stimulated by hormone treatment. *Journal of Endocrinology*, **114**, 319–324.

Martel, D., Monier, M.-N., Roche, D., De-Feo, V.J. and Psychoyos, A. (1989) Hormonal dependence of the metrial gland: further studies on oestradiol and progesterone receptor levels in the rat. *Journal of Endocrinology*, **120**, 465–472.

Martel, D., Monier, M.-N., Roche, D. and Psychoyos, A. (1991) Hormonal dependence of pinopode formation at the uterine luminal surface. *Human Reproduction*, **6**, 597–603.

Martin, L. (1964) The uptake of locally applied [6:7^{3}H] oestradiol-17β by the vagina of the ovariectomized mouse. *Journal of Endocrinology*, **30**, 337–346.

Martin, L. (1979) Early cellular changes and circular muscle contraction associated with the induction of decidualization by intrauterine oil in mice. *Journal of Reproduction and Fertility*, **55**, 135–139.

Martin, L. and Finn, C.A. (1968) Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. *Journal of Endocrinology*, **41**, 363–373.

Martin, L., Finn, C.A. and Carter, J. (1970) Effects of progesterone and oestradiol-17β on the luminal epithelium of the mouse uterus. *Journal of Reproduction and Fertility*, **21**, 461–469.

Martin, L., Finn, C.A. and Trinder, G. (1973) Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: an autoradiographic study. *Journal of Endocrinology*, **56**, 133–144.

Matthews, C.J., Adams, A.M. and Searle, R.G. (1985) Detection of macrophages and the characterisation of Fc receptor-bearing cells in the mouse decidua, placenta and yolk sac using the macrophage-specific monoclonal antibody F4/80. *Journal of Reproductive Immunology*, **7**, 315–323.

May, R. and Mead, R.A. (1986) Evidence for pineal involvement in timing implantation in the western spotted skunk. *Journal of Pineal Research*, **3**, 1–8.

Mayer, G. and Duluc, A.J. (1966) Homotransplantation et hétérotransplantation intratesticulaires chez le rat et la souris de blastocystes en phase de preimplantation et de lethargie. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, **263**, 1111–1114.

Mayer, G., Nilsson, O. and Reinius, S. (1967) Cell membrane changes of uterine epithelium and trophoblasts during blastocyst attachment in rat. *Zeitschrift fur Anatomie und Entwicklungsgeschichte*, **126**, 43–48.

Mead, R.A. (1968) Reproduction in western forms of the spotted skunk (*genus Spilogale*). *Journal of Mammalogy*, **49**, 373-390.

Mead, R.A. and McRae, M. (1982) Is estrogen required for implantation in the ferret? *Biology of Reproduction*, **27**, 540–547.

Mead, R.A., Concannon, P.W. and McRae, M. (1981) Effect of progestins on implantation in the western spotted skunk. *Biology of Reproduction*, **25**, 128–133.

Mead, R.A., Bremner, S. and Murphy, B.D. (1988) Changes in endometrial vascular permeability during the peri-implantation period in the ferret (*Mustela putorius*). *Journal of Reproduction and Fertility*, **82**, 293–298.

Medvei, V.C. (1982) *A History of Endocrinology*, MTP, Lancaster.

Mehrotra, S.N. and Finn, C.A. (1974) Cell proliferation in the uterus of the guinea pig. *Journal of Reproduction and Fertility*, **37**, 405–409.

Menke, T.M. and McLaren, A. (1970) Carbon dioxide production by mouse blastocysts during lactational delay of implantation or after ovariectomy. *Journal of Endocrinology*, **47**, 287–294.

Miller, B.G. (1973) Metabolism of RNA and pyrimidine nucleotides in the uterus during the early

decidual cell reaction. *Journal of Endocrinology*, **59**, 275–283.

Miller, B.G., Owen, W.H. and Emmens, C.W. (1968) The incorporation of tritiated uridine in the uterus and vagina of the mouse during early pregnancy. *Journal of Endocrinology*, **41**, 189–195.

Milligan, S.R. (1987) The sensitivity of the uterus of the mouse and rat to intraluminal instillation. *Journal of Reproduction and Fertility*, **79**, 251–259.

Milligan, S.R. and Finn, C.A. (1990) Failure of platelet-activating factor (PAF-acether) to induce decidualization in mice and failure of antagonists of PAF to inhibit implantation. *Journal of Reproduction and Fertility*, **88**, 105–112.

Milligan, S.R. and Lytton, F.D.C. (1983) Changes in prostaglandin levels in the sensitized and non-sensitized uterus of the mouse after the intra-uterine instillation of oil or saline. *Journal of Reproduction and Fertility*, **67**, 373–377.

Milligan, S.R. and Mirembe, F.M. (1984) Time course of the changes in uterine vascular permeability associated with the development of the decidual cell reaction in ovariectomized steroid-treated rats. *Journal of Reproduction and Fertility*, **70**, 1–6.

Milligan, S.R. and Mirembe, F.M. (1985) Intraluminally injected oil induces changes in vascular permeability in the 'sensitized' and 'non-sensitized' uterus of the mouse. *Journal of Reproduction and Fertility*, **74**, 95–104.

Milligan, S.R., Jaggers, D.C. and Patel, N. (1992) Is ornithine decarboxylase a good marker for the onset of decidualization? *Journal of Reproduction and Fertility*, Abstract Series No. 10, p 35.

Mitchell, B.S., Craggs, R.I. and Peel, S. (1980) Localisation of immunoglobulin (IgG) within the rat metrial gland. *Journal of Reproductive Immunology*, **2**, 235–244.

Mitchell, B.S., Craggs, R.I. and Peel, S. (1981) Localisation of immunoglobulin (IgG) in granulated metrial gland cells of deciduomata of the pseudopregnant rat. *Journal of Reproductive Immunology*, **3**, 237–241.

Moore, N.W. and Miller, B.G. (1976) Progesterone and oestrogen requirements for the survival of embryos in the ovariectomized ewe. *Journal of Reproduction and Fertility*, **46**, 536–537.

Mossman, H.W. (1937) Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Contributions to Embryology, Carnegie Institution, Washington*, **24**, 129–246.

Mossman, H.W. (1971) Orientation and site of attachment of the blastocyst: a comparative study, in *The Biology of the Blastocyst*, (ed. R.J. Blandau), University of Chicago Press, Chicago, pp. 49–57.

Moulton, B.C. (1974) Ovum implantation and uterine lysosomal enzyme activity. *Biology of Reproduction*, **10**, 543–548.

Moulton, B.C. and Ingle, C.B. (1981) Uterine lysosomal catshepsin D activity, rate of synthesis and immunochemical localization following initiation of decidualization in pseudopregnant rats. *Biology of Reproduction*, **25**, 393–398.

Mukku, V.R. and Stancel, G.M. (1985a) Regulation of epidermal growth factor receptors by estrogen. *Journal of Biological Chemistry*, **260**, 9820–9824.

Mukku, V.R. and Stancel, G.M. (1985b) Receptors for epidermal growth factor in the rat uterus. *Endocrinology*, **117**, 149–154.

Mulholland, J. and Leroy, F. (1989) Protein and MRNA synthesis in the peri-implantation rat endometrium, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 31–38.

Mulholland, J., Aplin, J.D., Ayad, S., Hong, L. and Glasser, S.R. (1992) Loss of collagen type VI from rat endometrial stroma during decidualization. *Biology of Reproduction*, **46**, 1136–1143.

Mullins, D.E., Horst, M.N., Bazer, F.W. and Roberts, R.M. (1980) Isolation and characterisation of a plasma membrane fraction derived from the luminal surface of the pig uterus during the oestrous cycle and early pregnancy. *Biology of Reproduction*, **22**, 1181–1192.

Mulnard, J.G. (1967) Les propriétés phagocytaires du trophoblaste au cours des premiéres phases de l'ovo-implantation chez la souris. *Archives de Biologie, Liege*, **78**, 575–594.

Murdoch, R.N., Kay, D.J. and Cross, M. (1978) Activity and subcellular distribution of mouse uterine alkaline phosphatase during pregnancy and pseudopregnancy. *Journal of Reproduction and Fertility*, **54**, 293–300.

Murphy, C.R. and Martin, B. (1985) Cholesterol in the plasma membrane of uterine epithelial cells: a freeze-fracture cytochemical study with digitonin. *Journal of Cell Science*, **78**, 163–172.

Murphy, C.R. and Rogers, A.W. (1981) Effects of ovarian hormones on cell membranes in the rat uterus. III. The surface carbohydrates at the apex of the luminal epithelium. *Cell Biophysics*, **3**, 305–320.

Murphy, C.R. and Turner, V.F. (1991) Glycocalyx carbohydrates of uterine epithelial cells increase

during early pregnancy in the rat. *Journal of Anatomy*, **177**, 109–115.

Murphy, C.R., Swift, J.E., Mukherjee, T.M. and Rogers, A.W. (1982a) The structure of tight junctions between uterine luminal epithelial cells at different stages of pregnancy. *Cell Tissue Research*, **223**, 281–286.

Murphy, C.R., Swift, J.F., Mukherjee, T.M. and Rogers, A.W. (1982b) Changes in the fine structure of the apical plasma membrane of endometrial epithelial cells during implantation in rats. *Journal of Cell Science*, **55**, 1–12.

Murphy, L.J. (1991) Estrogen induction of insulin-like growth factors and myc proto-oncogene expression in the uterus. *Journal of Steroid Biochemistry and Molecular Biology*, **40**, 223–230.

Murphy, L.J., Bell, G.E. and Friesen, H.G. (1987) Tissue distribution of insulin-like growth factor 1 and 2 messenger ribonucleic acid in the adult rat. *Endocrinology*, **120**, 1279–1282.

Myers, D.B., Clark, D.E. and Hurst, P.R. (1990) Decreased collagen concentration in rat uterine implantation sites compared with non-implantation tissue at days 6–11 of pregnancy. *Reproduction, Fertility and Development*, **2**, 607–612.

Nelson, W.O. and Pfiffner, J.J. (1930) Experimental production of deciduomata in the rat by an extract of the corpus luteum. *Proceedings of the Society of Experimental Biology and Medicine*, **27**, 863–866.

Nerquaye-Tetteh, J.O. and Clarke, J.R. (1987) Hormonal requirements and the influence of age on the decidual cell reaction in the bank vole (*Clethrionomys glareolus*). *Journal of Reproduction and Fertility*, **79**, 575–579.

Nieder, G.L. (1988) Effect of deciduogenic stimuli on protein secretion by the mouse uterus. *Journal of Reproduction and Fertility*, **84**, 273–281.

Nieder, G.L. and Macon, G.R. (1987) Uterine and oviductal protein secretion during early pregnancy in the mouse. *Journal of Reproduction and Fertility*, **81**, 287–294.

Nilsson, O. (1958) Ultrastructure of mouse uterine surface epithelium under different estrogenic influences. 2. Early effect of estrogen administered to spayed animals. *Journal of Ultrastructure Research*, **2**, 73–95.

Nilsson, O. (1966) Structural differentiation of luminal membrane in rat uterus during normal and experimental implantations. *Zeitschrift fur die Gesamte Anatomie*, **125**, 152–159.

Nilsson, O. (1967) Attachment of rat and mouse blastocysts onto uterine epithelium. *International Journal of Fertility*, **12**, 5–13.

Nilsson, O. (1972) Ultrastructure of the process of secretion in the rat uterine epithelium at implantation. *Journal of Ultrastructure Research*, **40**, 572–580.

Nilsson, O. (1974) Changes of the luminal surface of the rat uterus at blastocyst implantation, scanning electron microscopy and ruthenium red staining. *Zeitschrift fur Anatomie und Entwicklungsgeschichte*, **144**, 337–343.

Nilsson, O., Lindqvist, I. and Ronquist, G. (1974) Decreased surface change of mouse blastocyst at implantation. *Experimental Cell Research*, **83**, 421–423.

Norstedt, G., Leninovitz, A. and Eriksson, H. (1989) Regulation of uterine insulin-like growth factor I mRNA and insulin-like growth factor II mRNA by estrogen in the rat. *Acta Endocrinologica (Copenhagen)*, **120**, 466–472.

Novak, E. and Te Linde, R.W. (1924) The endometrium of the menstruating uterus. *Journal of the American Medical Association*, **83**, 900–906.

Noyes, R.W. and Dickman, Z. (1960) Relationship of ovular age to endometrial development. *Journal of Reproduction and Fertility*, **1**, 186–196.

Noyes, R.W., Hertig, A.T. and Rock, J. (1950) Dating the endometrial biopsy. *Fertility and Sterility*, **1**, 3–25.

Ober, W.B., Clyman, M.J., Decker, A. and Roland, M. (1964) Endometrial effects of synthetic progestagens. *International Journal of Fertility*, **9**, 597–608.

O'Grady, J.E., Moffat, G.E., McMinn, L., Vass, M., O'Hare, A. and Heald, P.J. (1975) Uterine chromatin template activity during the early stages of pregnancy in the rat. *Biochimica et Biophysica Acta*, **407**, 125–132.

Oliveira, S.F., Nagata, T., Abrahamsohn, P.A. and Zorn, T.M. (1991) Electron microscopic radioautographic study on the incorporation of [^{3}H]-proline by mouse decidual cells. *Cellular and Molecular Biolology*, **37**, 315–323.

O'Neill, C. (1985) Examination of the causes of early pregnancy associated thrombocytopenia in mice. *Journal of Reproduction and Fertility*, **73**, 567–577.

Orsini, M.W. (1963) Induction of deciduomata in hamster and rat by injected air. *Journal of Endocrinology*, **28**, 119–121.

Orsini, M.W. and Donovan, B.T. (1971) Implantation and induced decidualization of the uterus in the guinea pig, as indicated by

Pontamine Blue. *Biology of Reproduction*, **5**, 270–281.

Orsini, M.W. and McLaren, A. (1967) Loss of the zona pellucida in mice and the effect of tubal ligation and ovariectomy. *Journal of Reproduction and Fertility*, **13**, 485–479.

Orsini, M.W. and Meyer, R.K. (1959) Implantation of the castrate hamster in the absence of exogenous estrogen. *Anatomical Record*, **134**, 619–620.

Overlach, M. (1885) Die pseudomenstruirende mucosa uteri nach akuter phosphorvergiftung. *Archiv fur Mikroskopishche Anatomie*, **35**, 191.

Pampfer, S., Arceci, R.J. and Pollard, J.W. (1991) Role of colony stimulating factor-1 (CSF-1) and other lympho-hematopoietic growth factors in mouse pre-implantation development. *Bioessays*, **13**, 535–540.

Pampfer, S., Daiter, E., Barad, D. and Pollard, J.W. (1992) Expression of the colony-stimulating factor-1 receptor (c-fms proto-oncogene product) in the human uterus and placenta. *Biology of Reproduction*, **46**, 48–57.

Parkes, A.S., Dodds, E.C. and Noble, R.L. (1938) Interruption of early pregnancy by means of orally active oestrogens. *British Medical Journal*, **2**, 557–559.

Parr, E.L., Parr, M.B. and Young, J. D.-E. (1987) Localization of a pore-forming protein (Perforin) in granulated metrial gland cells. *Biology of Reproduction*, **37**, 1327–1335.

Parr, E.L., Young, L.H.Y., Parr, M.B. and Young, J.D.E. (1990) Granulated metrial gland cells of pregnancy mouse uterus are natural killer-like cells that contain perforin and serine esterase. *Journal of Immunology*, **145**, 2365–2372.

Parr, M.B. (1980) Endocytosis at the basal and lateral membranes of rat uterine epithelial cells during early pregnancy. *Journal of Reproduction and Fertility*, **60**, 95–99.

Parr, M.B. and Parr, E.L. (1974) Uterine luminal epithelium: protrusions mediate endocytosis not apocrine secretion in the rat. *Biology of Reproduction*, **11**, 220–233.

Parr, M.B. and Parr, E.L. (1977) Endocytosis in the uterine epithelium of the mouse. *Journal of Reproduction and Fertility*, **50**, 151–153.

Parr, M.B. and Parr, E.L. (1986) Effect of oestradiol-17β and progesterone on the number of plasma cells in uteri of ovariectomized mice. *Journal of Reproduction and Fertility*, **77**, 91–97.

Parr, M.B. and Parr, E.L. (1989) The barrier role of the primary decidual zone, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 163–170.

Paton, A.C. and Collins, W.P. (1992) Differentiation processes of granulosa cells, in *Oxford Reviews of Reproductive Biology*, Vol. 14, (ed. S. Milligan), Clarendon Press, Oxford, pp. 169–223.

Peach, R. and Bulmer, D. (1965) Cytochemistry and electron microscopy of the granulated metrial gland cells of the rat placenta. *Journal of Anatomy*, **99**, 415.

Peckham, B.M. and Greene, R.R. (1948) Prolongation of pseudopregnancy by deciduomata in the rat. *Proceedings of the Society of Experimental Biology and Medicine*, **69**, 417.

Peel, S. (1989) Granulated metrial gland cells. *Advances in Anatomy Embryology and Cell Biology*, **115**, 1–112.

Peel, S. and Bulmer, D. (1977) The fine structure of the rat metrial gland in relation to the origin of the granulated cells. *Journal of Anatomy*, **123**, 687–696.

Peel, S., Stewart, I.J. and Bulmer, D. (1983) Experimental evidence for the bone marrow origin of granulated metrial gland cells of the mouse uterus. *Cell and Tissue Research*, **233**, 647–656.

Perrota, C.A. (1962) Initiation of cell proliferation in vaginal and uterine epithelia of the mouse. *American Journal of Anatomy*, **111**, 195–204.

Perry, J.S. and Rowlands, I.W. (1962) Early pregnancy in the pig. *Journal of Reproduction and Fertility*, **4**, 175–188.

Persico, E., Scalona, M., Cicatiello, L., Sica, V., Bresciani, F. and Weisz, A. (1990) Activation of 'immediate-early' genes by estrogen is not sufficient to achieve stimulation of DNA synthesis in rat uterus. *Biochemical and Biophysical Research Communication*, **171**, 287–292.

Phelps, D. (1946) Endometrial vascular reactions and the mechanisms of nidation. *American Journal of Anatomy*, **79**, 167–197.

Phillips, C.A. and Poyser, N.L. (1981) Studies on the involvement of prostaglandins in implantation in rats. *Journal of Reproduction and Fertility*, **62**, 73–81.

Pollard, J.W. (1990) Regulation of polypeptide growth factor synthesis and growth-related gene expression in the rat and mouse uterus before and after implantation. *Journal of Reproduction and Fertility*, **88**, 721–731.

Pollard, J.W., Finn, C.A. and Martin, L. (1976)

Actinomycin D and uterine epithelial protein synthesis. *Journal of Endocrinology*, **69**, 161–162.

Pollard, J.W., Bartocci, A., Arceci, R., Orlofsky, A., Ladnes, M.B. and Stanley, E.R. (1987) Apparent role of the macrophage growth factor CSF-1 in placental development. *Nature (London)*, **330**, 484–486.

Pollard, J.W., Jahan, M. and Butterworth, P.J. (1990) Characterization and expression of uterine and placental alkaline phosphatases in the mouse. *Journal of Reproduction and Fertility*, **89**, 735–742.

Pollard, J.W., Hunt, J.S., Wiktor-Jedrzejczak, W. and Stanley, E.R. (1991) A pregnancy defect in the osteopetrotic (op/op) mouse demonstrates the requirement for CSF-1 in female fertility. *Developmental Biology*, **148**, 273–283.

Pollard, R.M. and Finn, C.A. (1972) Ultrastructure of the uterine epithelium during the hormonal induction of sensitivity and insensitivity to a decidual stimulus in the mouse. *Journal of Endocrinology*, **55**, 293–298.

Pollard, R.M. and Finn, C.A. (1974) Influence of the trophoblast upon differentiation of the uterine epithelium during implantation in the mouse. *Journal of Endocrinology*, **62**, 669–674.

Potter, S.W. and Morris, J.E. (1992) Changes in histochemical distribution of cell surface heparin sulphate proteoglycan in mouse uterus during the oestrous cycle and early pregnancy. *Anatomical Record*, **234**, 383–390.

Pouchet, F.-A. (1842) 'Théorie Positive de la Fécondation des Mammifères'. *Balliere et Fils, Paris*.

Prenant, L.A. (1898) De la valeur morphologique du corps jaune, son action physiologique et thérapeutique. *Revue Generale Science*, **9**, 646–650.

Prill, H.J. and Gotz, F. (1961) Blood flow in the myometrium and endometrium of the uterus. *American Journal of Obstetrics and Gynecology*, **82**, 102–108.

Psychoyos, A. (1960) La réaction deciduale est précédee de modifications précoces de la permeabilite capillaire de l'utérus. *Comptes Rendus Hebdomadaires des Séances et Memoires de la Société de Biologie, Paris*, **154**, 1384.

Psychoyos, A. (1961) Perméabilité capillaire et décidualisation utérine. *Comptes Rendus Hebdomadaires des Séances et Memoires de la Société de Biologie, Paris*, **252**, 1515.

Psychoyos, A. (1963) Précisions sur l'état de 'non-receptivite' de l'utérus. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris*, **257**, 1153–1156.

Psychoyos, A. (1974) Hormonal control of ovoimplantation. *Vitamins and Hormones*, **32**, 201–256.

Psychoyos, A. and Bitton-Casimiri, V. (1969) Captation *in vitro* d'un précurseur d'acide ribonucleique (ARN) (uridine-5-^{3}H) par le blastocyst du rat; différences entre blastocysts hormaux et blastocysts en diapause. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, **268**, 188–190.

Psychoyos, A. and Mandon, P. (1971) Étude de la surface de l'épithélium utérin au microscope électronique a balayage. Observation chez la rate au 4^{e} et 5^{e} jour de la gestation. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, **272**, 2723–2725.

Psychoyos, A. and Martel, D. (1985) Embryo-endometrial interactions at implantation, in *Implantation of the Human Embryo*, (eds R.G.E. Edwards, J.M. Purdy and P.C. Steptoe), Academic Press, London.

Pusey, J., Kelly, W.A., Bradshaw, J.M.C. and Porter, D.G. (1980) Myometrial activity and the distribution of the blastocysts in the uterus: interference by relaxin. *Biology of Reproduction*, **23**, 394–397.

Rachman, F., Bernard, O., Scheid, M.P. and Bennett, D. (1981) Immunological studies of mouse decidual cells. II. Studies of cells in artificially induced decidua. *Journal of Reproductive Immunology*, **3**, 41–48.

Rankin, J.C., Ledford, B.E. and Baggett, B. (1977) Early involvement of cyclic nucleotides in the artificially stimulated decidual cell reaction in the mouse uterus. *Biology of Reproduction*, **17**, 549–554.

Rankin, J.C., Ledford, B.E., Jonsson, H.T. and Baggett, B (1979) Prostaglandins, indomethacin and the decidual cell reaction in the mouse uterus. *Biology of Reproduction*, **20**, 399–404.

Rasweiler, J.J. (1979) Early embryonic development and implantation in bats. *Journal of Reproduction and Fertility*, **56**, 403–416.

Rasweiler, J.J.4th (1991) Spontaneous decidual reaction and menstruation in the black mastiff bat, *Molossus ater*. *American Journal of Anatomy*, **191**, 1–22.

Ravindranath, N. and Moudgal, N.R. (1989) Hormonal requirement for the establishment of pregnancy in primates, in *Blastocyst Implantation*,

(ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 195–200.

Reinius, S. (1967) Ultrastructure of blastocyst attachment in the mouse. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **77**, 257–266.

Reinius, S., Fritz, G.R. and Knobil, E. (1973) Ultrastructure and endocrinological correlates of an early implantation site in the rhesus monkey. *Journal of Reproduction and Fertility*, **32**, 171–173.

Reynolds, S.R.M. (1947) The physiologic basis of menstruation. A summary of current concepts. *Journal of the American Medical Association*, **135**, 552–557.

Reynolds, S.R.M. (1949) *The Physiology of the Uterus*, Noeber, New York.

Riddick, D.H. and Kusmik, W.F. (1977) Decidua: a possible source of amniotic fluid prolactin. *American Journal of Obstetrics and Gynecology*, **127**, 187–190.

Rider, V., McRae, A., Heap, R.B. and Feinstein, A. (1985) Passive immunization against progesterone inhibits endometrial sensitization in pseudopregnant mice and has antifertility effects which are reversible by steroid treatment. *Journal of Endocrinology*, **104**, 153–158.

Rider, V., Wang, M.-Y., Finn, C., Heap, R.B. and Feinstein, A. (1986) Antifertility effect of passive immunization against progesterone is influenced by genotype. *Journal of Endocrinology*, **108**, 117–121.

Rogers, P.A.W. and Gannon, B.J. (1981) The vascular and microvascular anatomy of the rat uterus during the oestrous cycle. *Australian Journal of Experimental Biology and Medical Science*, **59**, 667–679.

Rogers, P.A.W., Murphy, C.R. and Gannon, B.J. (1982a) Changes in the spatial organization of the uterine vasculature during implantation in the rat. *Journal of Reproduction and Fertility*, **65**, 211–214.

Rogers, P.A.W., Murphy, C.R. and Gannon, B.J. (1982b) Absence of capillaries in the endometrium surrounding the implanting rat blastocyst. *Micron*, **13**, 373–374.

Rogers, P.A.W. and Wischik, C.M. (1983) Cell organization in the stroma of the rat. II. Responses to progesterone in the ovariectomized rat. *Journal of Anatomy*, **137**, 541–553.

Romagnano, L. and Babiarz, B. (1990) The role of murine cell surface galactosyltransferase in trophoblast: laminin interactions *in vitro*. *Developmental Biology*, **141**, 254–261.

Rosenfeld, M.G. and Joshi, M.S. (1981) Effect of a rat uterine fluid endopeptidase on lysis of the zona pellucida. *Journal of Reproduction and Fertility*, **62**, 199–203.

Rothchild, I. and Meyer, R.K. (1942) Studies of the pretrauma factors necessary for placentoma formation in the rat. *Physiological Zoology*, **XV**, 216–223.

Ryan, G.B. and Majno, G. (1977) Acute inflammation. *American Journal of Pathology*, **86**, 184–276.

Rybo, G. (1966) Plasminogen activators in the endometrium. II. Clinical aspects. *Acta Obstetrica et Gynecologica Scandinavica*, **45**, 429–450.

Sachs, L. and Shelesnyak, M.C. (1955) The development and suppression of polyploidy in the developing and suppressed deciduoma in the rat. *Journal of Endocrinology*, **12**, 146–151.

Saksena, S.K., Lau, I.F. and Chang, M.C. (1976) Relationship between oestrogen, prostaglandin F_2 and histamine in delayed implantation. *Acta Endocrinologica*, **42**, 225–232.

Saksena, S.K., Watson, D.T., Lau, I.-F. and Shaikh, A.A. (1974) Peripheral plasma levels of E and F series prostaglandins during pseudopregnancy in the rat. *Prostaglandins*, **5**, 557–565.

Salamonsen, L.A., Nagase, H. and Woolley, D.E. (1991) Production of matrix metalloproteinase 3 (stromelysin) by cultured ovine endometrial cells. *Journal of Cell Science*, **100**, 381–385.

Salizar-Rubio, M., Gil Recansens, M.E., Hicks, J.J. and Gonzales-Angulo, A. (1980) High resolution cytochemical study of uterine epithelial surface of the rat at identified sites previous to blastocyst-endometrial contact. *Archivos de Investigaciones Medicas, Mexico*, **11**, 117–127.

Salvatore, C.A. (1968) Arteriole alterations in normal and hyperplastic endometrium. *American Journal of Obstetrics and Gynecology*, 493–500.

Samson, G.S. and Hill, J.P. (1931) Observations on the structure and mode of implantation of the blastocyst of Cavia. *Transactions of the Zoological Society*, **3**, 295–355.

Sanders, R.B., Bekairi, A.M., Abulaban, F.S. and Yochim, J.M. (1986) Uterine adenylate cyclase in the rat: Responses to a decidual-inducing stimulus. *Biology of Reproduction*, **35**, 100–105.

Saunders, J.R. (1966) Death in embryonic systems. *Science*, **154**, 604–612.

Saunderson, R. and Heald, P.J. (1974) Ornithine decarboxylase activity in the uterus of the rat during pregnancy. *Journal of Reproduction and Fertility*, **39**, 141–143.

Schindler, A.M., Bordingnon, P. and Bischof, P. (1984) Immunohistological localization of pregnancy-associated plasma protein-A in decidua and trophoblast. Comparison with HCG and fibrin. *Placenta*, **5**, 227–235.

Schlafke, S. and Enders, A.C. (1975) Cellular basis of interaction between trophoblast and uterus at implantation. *Biology of Reproduction*, **12**, 41–65.

Schlafke, S., Welsh, A.O. and Enders, A.C. (1985) Penetration of the basal lamina of the uterine luminal epithelium during implantation in the rat. *Anatomical Record*, **212**, 47–56.

Schmidt, I.G. (1943) Proliferation in the genital tract of the normal mature guinea pig treated with colchicine. *American Journal of Anatomy*, **73**, 59–80.

Schroder, R. (1915) Anatomishen studien zur normalen und pathologishchen physiologie des menstruationszyklus. *Archiv fur Gynaekologie*, **104**, 27–102.

Searle, R.F., Bell, S.C. and Billington, W.C. (1983) Ia antigen bearing decidual cells and macrophages in cultures of mouse decidual tissue. *Placenta*, **3**, 139–148.

Selye, H. and McKeown, T. (1935) Studies on the physiology of the maternal placenta of the rat. *Proceedings of the Royal Society*, **119**, 1–31.

Senanes, N. and Psychoyos, A. (1970) Effet de l'actinomycine-D sur le développement du déciduome chez la ratte. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris*, **271**, 430–433.

Serra, M.J., Ledford, B.E., Rankin, J.C. and Baggett, B. (1978) Changes in RNA polymerase activity in isolated mouse uterine nuclei during the decidual cell reaction. *Biochimica et Biophysica Acta*, **521**, 267–273.

Shapiro, M. and Talbert, G.B. (1969) Effect of maternal age upon decidualization (abstract). *Anatomical Record*, **163**, 261.

Shaw, K.J, Do, Y.C., Kjos, S., Anderson, P.W., Shinagawa, T., Dubeau, L. and Hsueh, W.A. (1989) Human decidua is a major source of renin. *Journal of Clinical Investigation*, **83**, 2985–2992.

Shaw, S.T. and Roche, P.C. (1980) Menstruation, in (ed. C.A. Finn), *Oxford Reviews of Reproductive Biology*, Clarendon Press, Oxford, pp. 41–96.

Shelden, R.M. (1973) Failure of ovarian steroids to influence blastocysts of weasels (*Mustela erminea*) ovariectomized during delayed implantation. *Endocrinology*, **92**, 638–641.

Shelesnyak, M.C. (1931) The induction of pseudopregnancy in the rat by means of electrical stimulation. *Anatomical Record*, **49**, 179–183.

Shelesnyak, M.C. (1957) Some experimental studies on the mechanisms of ova-implantation in the rat. *Recent Progress in Hormone Research*, **13**, 269–322.

Shelesnyak, M.C. (1960) Nidation of the fertilized ovum. *Endeavour*, **19**, 81–86.

Simmen, F.A. and Simmen, R.C. (1991) Peptide growth factors and proto-oncogenes in mammalian conceptus development. *Biology of Reproduction*, **44**, 1–5.

Slotta, K.H., Ruschig, H. and Fells, E. (1934) Ulber die hormone des corpus luteum. *Helvetica Chimica Acta*, **17**, 1361–1362.

Smith, D.M. (1968) The effect on implantation of treating cultured mouse blastocysts with oestrogen *in vitro* and the uptake of [^{3}H] oestradiol by blastocysts. *Journal of Endocrinology*, **41**, 17–29.

Smith, L.J. (1966) Metrial gland and other glycogen containing cells in the mouse uterus following mating and through implantation of the embryo. *American Journal of Anatomy*, **119**, 15–24.

Smith, P.E. and Engle, E.T. (1932) Prevention of experimental uterine bleeding in Macacus monkey by corpus luteum extract (progestin). *Proceedings of the Society of Experimental Biology and Medicine*, **29**, 1225–1227.

Solomkin, J.S. and Simmons, R.L. (1983) Cellular and subcellular mediators of acute inflammation. *Surgical Clinics of North America*, **63**, 225–243.

von Spee (1893) Beitrag zue Entweickelungsgeschichte der fruheren Stadien des Meerschweinchens bis zue Vollendung der Keimblase. *Archiv fur Anatomie und Physiologie*, **7**, 44–60.

Spinks, N.R. and O'Neill, C. (1988) Antagonists of embryo-derived platelet-activating factor prevent implantation of mouse embryos. *Journal of Reproduction and Fertility*, **84**, 89–98.

Stancel, G.M., Baker, V.V., Hyder, S.M., Kirkland, J.L. and Loose-Mitchell, D.S. (1993) Oncogenes and uterine function, in (ed. S. Milligan), *Oxford Reviews of Reproductive Biology*, Vol 15, Clarendon Press Oxford, (in press)

Staples, L.D., Cummings, I.A., Lawson, R.A.S. and Morgan, F.J. (1976) Proteins of the uterine secretion in sheep. *Journal of Reproduction and Fertility*, **46**, 532.

Staples, L.D., Heap, R.B., Wooding, F.B.P. and King, G.J. (1983) Migration of leucocytes into the uterus after acute removal of ovarian pro-

gesterone during early pregnancy in the sheep. *Placenta*, **4**, 339–350.

Steer, H.W. (1970) The trophoblastic knobs of the preimplanted rabbit blastocyst: a light and electron microscopic study. *Journal of Anatomy*, **107**, 315–325.

Steer, H.W. (1971) Implantation of the rabbit blastocyst: the adhesive phase of implantation. *Journal of Anatomy*, **109**, 215–227.

Stewart, C.L., Kaspar, P., Burnet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*, **359**, 76–79.

Stewart, I. and Peel, S. (1977) The structure and differentiation of granulated metrial gland cells of the pregnancy mouse uterus. *Cell and Tissue Research*, **184**, 517–527.

Stewart, I. and Peel, S. (1978) The differentiation of the decidua and the distribution of metrial gland cells in the pregnant mouse uterus. *Cell and Tissue Research*, **187**, 167–179.

Stiles, C.D. (1985) The biological role of oncogenes – insights from platelet-derived growth factor. *Cancer Research*, **45**, 5215–5218.

Stimson, W.H., Farquharson, D.M. and Lang, G.D. (1983) Pregnancy-associated α_2-macroglobulin – a new serum protein elevated in normal human pregnancy. *Journal of Reproductive Immunology*, **5**, 321–327.

Stockdale, F.E. and Topper, Y.J. (1966) The role of DNA synthesis and mitosis in hormone dependent differentiation. *Proceedings of the National Academy of Sciences, of the USA*, **56**, 1283–1289.

Strickland, S., Reich, E. and Sherman, M.I. (1976) Plasminogen activator in early embryogenesis: enzyme production by trophoblast and parietal endoderm. *Cell*, **9**, 231–240.

Sutherland, A.E., Calarco, P.G. and Damsky, C.H. (1988) Expression and function of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. *Journal of Cell Biology*, **106**, 1331–1348.

Svalander, P.C., Odin, P., Nilsson, B.O. and Obrink, B. (1987) Trophectoderm surface expression of the cell adhesion molecule cell-CAM 105 on rat blastocysts. *Development*, **100**, 653–660.

Svalander, P.C., Odin, P., Nilsson, B.O. and Obrink, B. (1990) Expression of cell-CAM 105 in the apical surface of rat uterine epithelium is controlled by ovarian steroid hormones. *Journal of Reproduction and Fertility*, **88**, 213–221.

Swift, A.D. and O'Grady, J.E. (1976) Uterine cyclic AMP in early pregnancy in the rat. *Journal of Endocrinology*, **68**, 35.

Tabor, H. and Tabor, C.W. (1972) Biosynthesis and metabolism of 1:4 diaminobutane, spermidine, spermine and related amines. *Annual Review of Enzymology*, **36**, 203–268.

Tachi, C., Tachi, S. and Lindner, H.R. (1970) Action of antihistaminics on the endometrium and the histamine theory of decidual induction. *Journal of Reproduction and Fertility*, **23**, 169–172.

Tachi, C., Tachi, S. and Lindner, H.R. (1974) Effect of ovarian hormones upon nucleolar ultrastructure in endometrial stromal cells of the rat. *Biology of Reproduction*, **10**, 404–413.

Tafani, A. (1886) Sulle condizioni utero-placentari della vita fetale. *Archivo della Scuola d'Anatomie Pathologia*, **4**, 52.

Takemori, K., Okamura, H., Kanzaki, H., Koshida, M. and Konishi, I. (1984) Scanning electron microscopy study on corrosion cast of rat uterine vasculature during the first half of pregnancy. *Journal of Anatomy*, **138**, 163–173.

Tamada, H., Das, S.K., Andrews, G.K. and Dey, S.K. (1991) Cell-type-specific expression of transforming growth factor-α in the mouse uterus during the peri-implantation period. *Biology of Reproduction*, **45**, 365–372.

Tawia, S.A. and Rogers, A.W. (1992) *In vivo* microscopy of the subepithelial capillary plexus of the endometrium of rats during embryo implantation. *Journal of Reproduction and Fertility*, **96**, 673–680.

Tawfik, O.W. and Dey, S.K. (1988) Further evidence for role of leukotrienes as mediators of decidualization in the rat [published erratum appears in *Prostaglandins* (1988) 36:747] *Prostaglandins*, **35**, 379–386.

Tekelioglu-Uysal, M., Edwards, R.G. and Kisnisci, H.A. (1975) Ultrastructural relationship between decidua, trophoblast and lymphocytes at the beginning of human pregnancy. *Journal of Reproduction and Fertility*, **42**, 431–438.

Terranova, P.F. (1980) Relationship between the decidual response and ovarian steroidogenesis in the unilaterally ovariectomized pseudopregnant rat. *Journal of Endocrinology*, **85**, 525–531.

Tobert, J.A. (1976) A study of the possible role of the prostaglandins in decidualization using a non-surgical method for the instillation of fluids into the rat uterine lumen. *Journal of Reproduction and Fertility*, **47**, 391–393.

Tomita, K., McCoshen, J.A., Fernandez, C.S. and

Tyson, J.E. (1982) Immunologic and biologic characteristics of human decidual prolactin. *American Journal of Obstetrics and Gynecology*, **142**, 420–426.

Trotula of Salerno C 11 *The Diseases of Women*. Translated by Elizabeth Mason-Hohl, Los Angeles, Ward Ritchie Press, 1940

Tsang, B.K. and Ooi, T.C. (1982) Prostaglandin secretion by human endometrium *in vitro*. *American Journal of Obstetrics and Gynecology*, **142**, 626–633.

Turner, W. (1873) Report on the progress of anatomy. *Journal of Anatomy and Physics*, **8**, 159–178.

Turner, W. (1876) Lectures on Comparative Anatomy of the Placenta. University of Edinburgh.

Vokaer, R. (1952) Recherches histophysiologiques sur l'endomètre du rat, en particulier sur le conditionnement hormonal de ses propriétés athrocytaires. *Archives de Biologie*, **63**, 3–84.

Vokaer, R. and Leroy, F. (1962) Experimental study on local factors in the process of ovoimplantation in rats. *American Journal of Obstetrics and Gynecology*, **83**, 141–148.

Wade, H. and Watson, B.P. (1908) The anatomy and histology of an early tubal gestation. *Journal of Obstetrics and Gynaecology of the British Empire*, **14**, 89–99.

van Wagenen, G. and Aberle, S.B.D. (1931) Menstruation in Pithecus (Macacus) rhesus following bilateral and unilateral ovariectomy performed early in the cycle. *American Journal of Physiology*, **99**, 271–278.

Waites, G.T. and Bell, S.C. (1984) Identification of a murine pregnancy-associated serum protein (α_1-PAP) as a female-specific acute-phase reactant. *Journal of Reproduction and Fertility*, **70**, 581–589.

Waites, G.T.and Bell, S.C. (1986) Pregnancy proteins and the acute-phase response, in *Pregnancy Proteins in Animals*, (ed. J. Hau), Walter de Gruyter, Berlin, New York, pp. 195–212.

Waites, G.T. and Bell, S.C. (1989) Immunohistological localization of human pregnancy-associated endometrial α_2-globulin (α_2-PEG), a glycosylated B-lactoglobulin homologue, in the decidua and placenta during pregnancy. *Journal of Reproduction and Fertility*, **87**, 291–300.

Warren, K.S. and Kellermeyer, R.W. (1968) The foreign body granuloma as a response to chemical mediators of inflammation (abstract). *Journal of Clinical Investigation*, **47**, 99a.

Wathes, D.C. and Wooding, F.B.P. (1980) An electron microscope study of implantation in the cow. *American Journal of Anatomy*, **159**, 285–306.

Webb, F.T.G. (1975) Implantation in ovariectomized mice treated with dibutyryl adenosine 3', 5'-monophosphate (dibutyryl cyclic AMP). *Journal of Reproduction and Fertility*, **42**, 511–517.

Weichert, C.K. (1928) Production of placentomata in normal and ovariectomized guinea pigs and albino rats. *Proceedings of the Society of Experimental Biology and Medicine*, **25**, 490–491.

Weill, P. (1921) Les cellules granuleuses des muqueuses intestinale et utérine. *Archives d'Anatomie Microscopique*, **17**, 77–82.

Weisz, A. and Bresciani, F. (1988) Estrogen induces expression of c-fos and c-myc protooncogenes in rat uterus. *Molecular Endocrinology*, **2**, 816–824.

Weisz, A., Cicatiello, L., Persico, E., Scalona, M. and Bresciani, F. (1990) Estrogen stimulates transcription of c-jun protooncogene. *Molecular Endocrinology*, **4**, 1041–1050.

Weitlauf, H.M. (1973) *In vitro* uptake and incorporation of amino acids by blastocysts from intact and ovariectomized mice. *Journal of Experimental Zoology*, **183**, 303–308.

Weitlauf, H.M. (1978) Factors in mouse uterine fluid that inhibit the incorporation of [^{3}H] uridine by blastyocysts *in vitro*. *Journal of Reproduction and Fertility*, **52**, 321–325.

Weitlauf, H.M. (1985) Changes in the rate of translation with reactivation of delayed implanting mouse embryos. *Journal of Experimental Zoology*, **236**, 309–312.

Weitlauf, H.M. (1989) Embryonic signaling at implantation in the mouse. *Progress in Clinical and Biological Research*, **294**, 359–376.

Weitlauf, H.M. and Suda-Hartman, M. (1988) Changes in secreted uterine proteins associated with embryo implantation in the mouse. *Journal of Reproduction and Fertility*, **84**, 539–549.

Welsh, A.O and Enders, A.C. (1989) Comparisons of the ability of cells from rat and mouse blastocysts and rat uterus to alter complex extracellular matrix *in vitro*, in *Blastocyst Implantation*, (ed. K. Yoshinaga), Serono Symposia, Adams, Boston, USA, pp. 55–74.

Wewer, U.M., Damjanov, A., Weiss, J., Liotta, L.A. and Damjanov, I. (1986) Mouse endometrial stromal cells produce basement-membrane components. *Differentiation*, **32**, 49–58.

Wheeler, A.G., Hurst, P.R., Poyser, N.L. and Eckstein, P. (1983) Uterine histology and prostaglandin concentration and utero-ovarian venous

steroid and prostaglandin concentrations during the luteal phase of the menstrual cycle in baboons (*Papio spp*) with or without a IUD. *Journal of Reproduction and Fertility*, **67**, 35–46.

Whyte, A. and Allen, W.R. (1985) Equine endometrium at pre-implantation stages of pregnancy has specific glycosylated regions. *Placenta*, **6**, 537–542.

Williams, K.I. and Downing, I. (1977) Prostaglandin and thromboxane production by rat decidual microsomes. *Prostaglandins*, **14**, 813–817.

Williams, M.F. (1948) The vascular architecture of the rat uterus as influenced by estrogen and progesterone. *American Journal of Anatomy*, **83**, 247–307.

Williams, T.J. (1979) Prostaglandin E_2, prostaglandin I_2 and the vascular changes in inflammation. *British Journal of Pharmacology*, **65**, 517–524.

Wilson, E.W. (1969) Alkaline phosphatase in predecidual cells of the human endometrium. *Journal of Reproduction and Fertility*, **19**, 567–568.

Wilson, I.B. (1960) Implantation of tissue transplants in the uteri of pseudopregnant mice. *Nature*, **185**, 553–554.

Wilson, I.B. (1963) A tumour tissue analogue of the implanting mouse embryo. *Proceedings of the Zoological Society of London*, **141**, 137–151.

Wimsatt, W.A. (1963) Delayed implantation in the Ursidae, with particular reference to the black bear (*Ursus americanus Pallus*), in *Delayed Implantation*, (ed. A.C. Enders), University Press, Chicago.

Wimsatt, W.A. (1975) Some comparative aspects of implantation. *Biology of Reproduction*, **12**, 1–40.

Winterhager, E. and Kuhnel, W. (1982) Alterations in intercellular junctions of the uterine epithelium during the preimplantation phase in the rabbit. *Cell and Tissue Research*, **224**, 517–526.

Winterhager, E., Busch, L.C. and Kuhnel, W. (1984) Membrane events involved in fusion of uterine epithelial cells in pseudopregnant rabbits. *Cell and Tissue Research*, **235**, 357–363.

Winterhager, E., Stutenkemper, R., Traub, O., Beyer, E. and Willecke, K. (1991) Expression of different connexin genes in rat uterus during decidualization and at term. *European Journal of Cell Biology*, **55**, 133–42.

Wischik, C.A. and Rogers, P.A.W. (1982) Cell organization in the stroma of the rat uterus. I. The ovariectomized rat. *Journal of Anatomy*, **135**, 707–718.

Wislocki, G.B., Weiss, L.P., Burgos, M.H. and Ellis, R.A. (1957) The cytology, histochemistry and electron microscopy of the granular cells of the metrial gland of the gravid rat. *Journal of Anatomy*, **91**, 13–140.

Wood, G.W. (1980) Immunohistochemical identification of macrophages in murine placentae, yolk-sac membranes and pregnant uteri. *Placenta*, **1**, 309–317.

Wood, G.W., De, M., Sanford, T. and Choudhuri, R. (1992) Macrophage colony stimulating factor controls macrophage recruitment to the cycling mouse uterus. *Developmental Biology*, **152**, 336–343.

Wood, J.G. and Barley, V.L. (1970) Biochemical changes in forming and regressing deciduoma in the rat uterus. *Journal of Reproduction and Fertility*, **23**, 469–475.

Wooding, F.B.P., Staples, L.D. and Peacock, M.A. (1982) Structure of trophoblastic papillae on the sheep conceptus at implantation. *Journal of Anatomy*, **134**, 507–516.

Wooding, F.B.P. and Wathes, D.C. (1980) Binucleate cell migration in the bovine placentome. *Journal of Reproduction and Fertility*, **59**, 425–430.

Wordinger, R.J. and Amsler, K.K. (1980) Histochemical identification of the glycocalyx layer in the bovine oviduct and endometrium. *Animal Reproduction Science*, **3**, 189–193.

Wordinger, R.J., Jackson, F.L. and Morrill, A. (1986) Implantation, deciduoma formation and live births in mast cell-deficient mice (w/w^v). *Journal of Reproduction and Fertility*, **77**, 471–476.

Wordinger, R.J., Moss, A.E., Lockard, T., Gray, D., Chang, I.-F.C. and Jackson, T.L. (1992) Immunohistochemical localization of basic fibroblast growth factor within the mouse uterus. *Journal of Reproduction and Fertility*, **96**, 141–152.

Wu, J.T. and Chang, M.C. (1973) Hormonal requirement for implantation and embryonic development in the ferret. *Biology of Reproduction*, **9**, 350–355.

Wu, J.T. and Chang, M.C. (1977) Failure of dibutyryl cyclic AMP to induce implantation in rats and mice. *Biology of Reproduction*, **17**, 355–360.

Wu, J.T. and Chang, M.C. (1978) Increase in concanavalin A binding sites in mouse blastocysts during implantation. *Journal of Experimental Zoology*, **205**, 447–453.

Wu, J.T. and Meyer, R.K. (1970) The effect on implantation of culturing delayed rat blastocysts in medium containing 17β oestradiol. *Biology of*

Reproduction, **3**, 201–204.

Yamamoto, Y., Kurohmaru, M. and Hayashi, Y. (1992) Localization of type 1 interferon in murine trophoblast and decidua during decidual formation. *Journal of Reproduction and Fertility*, **95**, 559–565.

Yee, G.M. and Kennedy, T.G. (1988) Stimulatory effects of prostaglandins upon endometrial alkaline phosphatase activity during the decidual cell reaction in the rat. *Biology of Reproduction*, **38**, 1129–1136.

Yee, G.M. and Kennedy, T.G. (1991) Role of cyclic adenosine 3′, 5′-monophosphate in mediating the effect of prostaglandin E_2 on decidualization *in vitro*. *Biology of Reproduction*, **45**, 163–171.

Ziegler (1843) Beobachtungen Uber Die Brunst und den Embryo der Rehe, Hanover.

Zorn, T.M., Bijovsky, A.T., Bevilacqua, E.M. and Abrahamsohn, P.A. (1989) Phagocytosis of collagen by mouse decidual cells. *Anatomical Record*, **225**, 96–100.

Zybina, E.V. and Grishenko, T.A. (1972) Spectrophotometrical estimation of ploidy level in decidual cells of the endometrium of the white rat (In Russian). *Doklady Akademii Nauk (SSSR)*, **14**, 284–290.

PLACENTATION 4

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4.1 INTRODUCTION

This review of vertebrate placental structure attempts to integrate some of the considerable body of work done using electron microscopic and other techniques since Amoroso's classic 1952 chapter. The reference list concentrates on the most recent work. Mossman (1987) provides the best comprehensive list of placental references. This chapter is mainly concerned with providing data to facilitate broad comparisons of structures and secretions both during development and in their final form with a view to relating them to the functional activity of the placenta. The remarkably wide variety of structures in the mature placenta develops from a basically similar repertoire of extraembryonic membranes as typified in the amniote egg, and the diversity can be further simplified by use of straightforward structural criteria. The major problem is to relate those criteria to the wide range of functions served by the placenta. A few non-amniotes have developed analogous structures which serve similar functions to the extraembryonic membranes, and these offer further insights into the requirements for successful fetal development (Hagan, 1951; Woollacott and Zimmer, 1975; Amoroso *et al.*, 1979; Bone *et al.*, 1985).

The placenta developed as an organ of physiological exchange to take advantage of the protection and egg economy provided by the adoption of viviparity. The initially small area of fetomaternal apposition is usually enormously increased by folding and refolding as it proliferates in parallel with the growth of the fetus. The main functions of the placenta are to act as a surrogate fetal lung, gut and kidney and to camouflage the usually allogeneic fetus from the mother's immune system (Lala *et al.*, 1983; Gill and Wegmann, 1987).

The surrogacy requires efficient exchange of a necessarily wide range of metabolites and hormones using many different types of transport processes (passive diffusion, active transport, pinocytosis and phagocytosis), all favoured by a reduction in the separation of fetal and maternal blood flows (Moll, 1985; Munro, 1986).

The immunological camouflage is vital to avoid recognition by the multifactorial array of cellular and hormonal mechanisms mediating sensitization and immune rejection and is best served by the provision of a barrier between the two circulations (Amoroso and Perry, 1975).

Since the surrogacy and camouflage requirements are so complex and mutually antagonistic (it being very difficult to adequately conceal and feed an invading cellular army) it is perhaps not surprising that such a wide variety of structures has evolved as solutions to these two multifaceted major problems. In addition, a system for rapid disposal of the placenta with minimum trauma is essential and there are constraints of gross size, habitat (arboreal gliding versus aquatic diving) and lifestyle (secure den versus run-as-soon-as-born) further modifying the requirements defining a successful placenta.

Perhaps because of these various and usually conflicting pressures, there seems to be no straightforward progression to a unique 'best', most efficient placenta in parallel with the generally accepted course(s) of evolution of placental animals. Different groups of mammals have evolved different structural solutions, all originally from a common pattern of membranes modified by a general trend to a reduction in separation between fetal and maternal circulations. The greater the understanding of these structural solutions the easier it should be to design experiments to elucidate the manifold functions of the placenta, to establish how best to judge the 'efficiency' of a placenta and to plan strategies to reduce the considerable prenatal embryo losses (Wilmut *et al.*, 1986). Many of these functions are modified by hormones and their role

is discussed in section 4.4 on placental endocrinology.

4.2 GENERAL CHARACTERISTICS AND DEFINITIONS

4.2.1 PLACENTA

The most comprehensive definition is that of Mossman (1937): 'an apposition of parental, (usually maternal) and fetal tissue for the purposes of physiological exchange'. However, Steven's suggestion of 'an arrangement of one or more transporting epithelia between fetal and maternal circulations' more vividly encapsulates the functional aspects.

Mossman's definition is wide enough to cover the situations found in a variety of non-amniotes: tunicates, seahorses, some insects, fishes and amphibians as well as the amniotes (reptiles and mammals). The degree of apposition *in vivo* is sometimes difficult to establish because of technical problems of fixation and tissue preparation. There is good reason to believe that it is very close, often including microvillar interdigitation between fetal and maternal epithelia in the amniotes, but a shell or shell membrane remnant may persist in most reptiles and some fish (sections 4.3.1 and 4.3.4).

The tissue on the maternal side of the apposition is usually a derivative of either ovarian, oviductal or uterine epithelium or connective tissue.

The fetal component is invariably a derivative of the cellular ectodermal epithelium (trophoblast) which formed the blastocyst surface. This epithelium together with internal membranes of the conceptus eventually forms the choriovitelline and chorioallantoic placental structures.

Physiological exchange covers a range of transport processes from simple diffusion of O_2 and CO_2 to the uptake of uterine secretions and maternal red blood cells. There is increasing evidence that mature placentas have different regions for the different forms of uptake (section 4.2.5a) and also that different types of placenta have evolved different methods for the uptake of particular nutrients (e.g. iron, see section 4.2.3f).

4.2.2 EMBRYONIC MEMBRANES

The extraembryonic membranes of the vertebrate conceptus form from, and are in continuity with, the three germ layers (ectoderm, mesoderm and endoderm) of the embryo. They provide the basis for the placental structures essential for the physiological maintenance and protection of embryos within the egg or uterus (Figure 4.1; Mossman, 1937, 1987; Steven, 1975a).

(a) Yolk sac

This is formed by the enclosure of the yolk initially by cells from the embryonic disc which form a single cellular layer of ectoderm (unilaminar yolk sac, vitelline sac or omphalopleur) (Figure 4.1). Subsequent migration of a layer of endoderm internal to the ectoderm forms a bilaminar yolk sac, and finally growth of mesoderm between the first two produces a trilaminar yolk sac. If no yolk is present the last two membranes develop in the same way after a hollow unilaminar blastocyst is produced from the solid ball of cells forming the morula.

In the fishes and amphibians the mesoderm of the trilaminar yolk sac forms the blood vessels which vascularize the yolk sac and transport its contents to the growing embryo. This vascularized yolk sac persists in some fishes after the release of the embryo from the investments of the egg. Retention of the developing embryo within the mother allows the possibility of modifying the vascularized yolk sac into an organ for direct fetomaternal exchange. Several of the cartilaginous fishes (elasmobranchs – skates and rays) have developed such a placental system whereas bony fishes (Osteichthyes) and amphibia have modified other parts of their

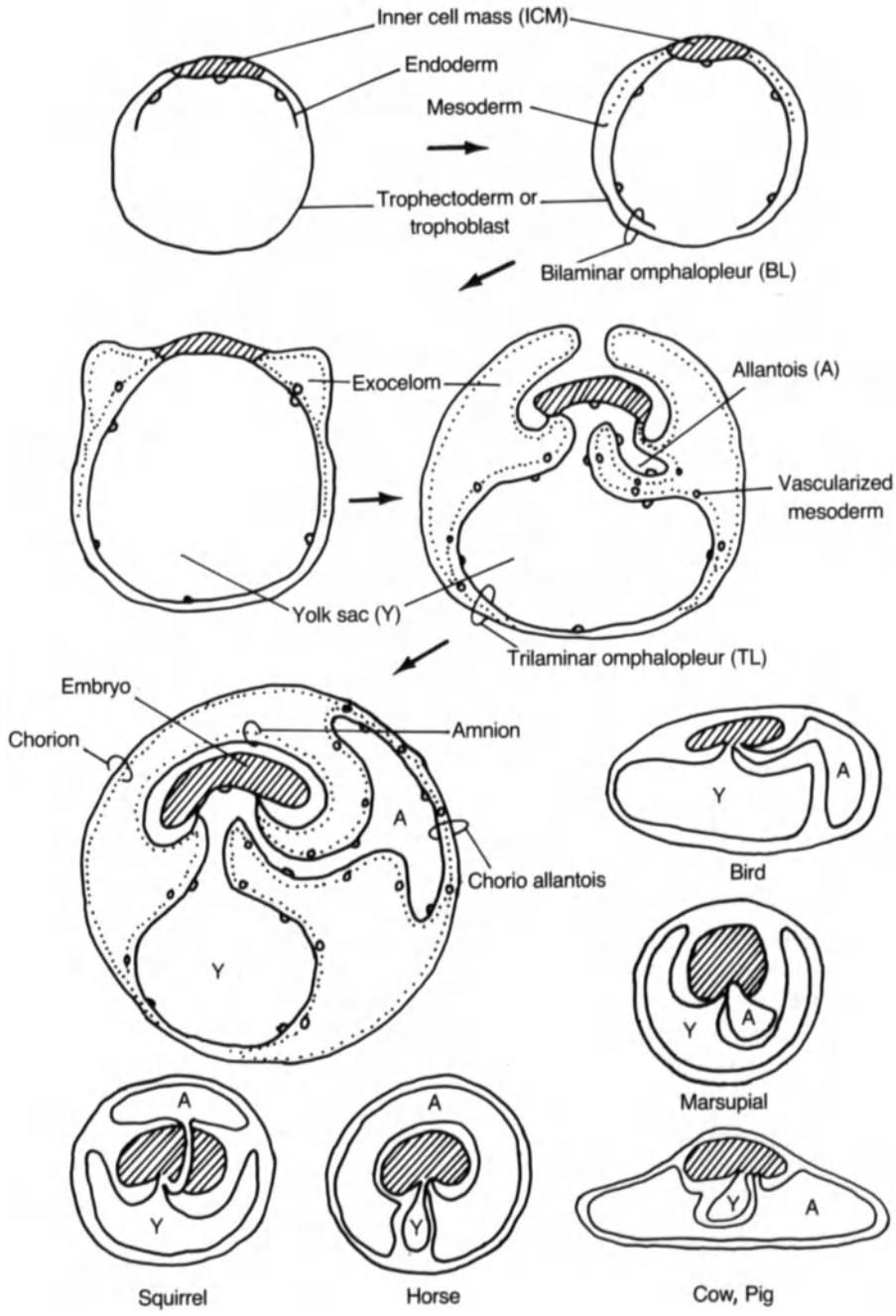


Figure 4.1 Development of the basic extraembryonic membranes with diagrams of the definitive disposition of yolk sac and allantois in various animals.

body surfaces for the same purpose (section 4.3.2).

In reptiles and mammals the initial spread of the mesoderm converts only the top part of the bilaminar yolk sac into the vascular trilaminar form. This is the basis for the development of the other extraembryonic membranes typical of the amniotes: chorion, amnion and allantois (Figure 4.1).

(b) Chorion, amnion and allantois

Growth of these membranes starts when the mesoderm of the partly trilaminar yolk sac splits to form the exocoelom in continuity with the embryonic coelom (Figure 4.1). The subsequent development of the mesoderm depends on whether it is associated with ectoderm or endoderm. Mesoderm plus endoderm (gut wall, splanchnopleur = definitive yolk sac wall) vascularizes very readily, whereas mesoderm plus ectoderm (body wall, somatopleur = chorion) only rarely produces blood vessels.

This chorion (ectoderm plus nonvascular mesoderm) produces folds which enclose the embryo to form the fluid-filled amniotic sac (Figure 4.1). Inside this the embryo can develop free of asymmetric constraints, in a shock-absorbing and compression-resistant environment. The amniotic membrane is thus derived directly from the chorion. In a few animals the amnion forms by cavitation (see Figure 4.2, rat and mouse).

As the amniotic folds fuse an endodermal vesicle grows from the hind end of the embryonic gut to form the allantoic vesicle, covered from its inception by vascular splanchnopleuric mesoderm. The allantois is in close anatomical association with the developing urogenital system of the embryo and acts as a site for waste deposition. Its rich blood supply also allows it to develop as an organ of respiratory exchange when it grows through the exocoelom to fuse with the chorion which surrounds the embryo (Figures 4.1, 4.2 and 4.75).

(c) Variations on the basic system

These membrane developments provide the basic systems which are common to all amniotes and can usually be recognized at least at the earlier stages of placental growth. The band of vascularized yolk sac fused with the ectoderm forms the fetal basis for the choriovitelline placenta and the vascularized allantois plus chorion forms the fetal part of the chorioallantoic placenta. Both are functional through most of prenatal development in reptiles, monotremes and marsupials (Figures 4.16 and 4.17).

Choriovitelline placenta

In eutherian mammals the choriovitelline placenta functions only for a short time and varies considerably in extent in different orders. In carnivores and some ungulates it may form an extensive area in early pregnancy, but eventually the vascularized yolk sac is completely separated from the chorion by expansion of the exocoelom. In most rodents, lagomorphs and insectivores formation of the trilaminar choriovitelline placenta, if it occurs at all, is restricted by the persistence of a large bilaminar segment forming the lower half of yolk sac and conceptus. A very different sort of yolk sac placenta is formed subsequently when expansion of the exocoelom invaginates the vascularized embryonic (top) half of the yolk sac into the bilaminar abembryonic (bottom) half to provide a multilayered cup apposed to the endometrium. The yolk sac cavity is reduced to a mere slit and the resultant layers referred to as an 'incompletely inverted yolk sac placenta' (Figures 4.2 and 4.3).

Loss of the non-vascularized bilaminar yolk sac layers adjacent to the endometrium produces complete inversion (Figures 4.2 and 4.3). What is 'inverted' is the sequence of tissues at the maternofetal interface. The choriovitelline placenta has maternal tissue: fetal ectoderm, mesoderm, endoderm; the

inverted yolk sac placenta has maternal tissue: fetal endoderm, mesoderm.

The choriovitelline placenta is usually transitory, but the inverted yolk sac type may persist to term and form part of the wide range of mature placental structures developed from the extraembryonic membranes. For example, the ungulates have a mere relic of a yolk sac but an enormous chorioallantois; the marsupials retain a large functional trilaminar choriovitelline placenta with a persistent bilaminar area and an allantois that does not usually reach the chorion; some insectivores and rodents have both inverted yolk sac and chorioallantoic placentas which function up to parturition (Figures 4.1, 4.2 and 4.3). The type of implantation can also greatly affect extraembryonic membrane development.

Implantation and membrane development

Implantation of the blastocyst, the initial apposition of fetal and maternal surfaces, usually occurs over the entire uterine luminal surface. However, some animals have very small blastocysts which develop in a narrow endometrial cleft (mouse, rat) or actively invade the endometrium (guinea pig, man: referred to as interstitial implantation) (section 4.2.9).

Presumably, because of lack of space around the conceptus, the development of the embryonic membranes is considerably modified in species with interstitial implantation although the basic pattern described above can be recognized if sufficient stages are available. The major differences are that part of the original outer ectodermal layer may be lost at a very early stage; the amniotic sac forms by cavitation in a solid mass of cells rather than by folding; and the allantoic endodermal vesicle may be very small or absent so that the allantois is replaced by a solid rod of mesoderm. Development of the placental membranes is compressed in both time and space and the homologies of the membranes can be appreciated best from comparative diagrams (Figure 4.2).

4.2.3 TYPES OF CHORIOALLANTOIC PLACENTATION

(a) Introduction

The great diversity among chorioallantoic placental structures has led to many attempts to devise a classification which would categorize only those common structural features which may be used as a valid basis for evolutionary comparisons and experimental investigations of function. No classification scheme based on a single criterion has proved adequate and the current practice is to use several in parallel, which produces convenient and possibly instructive groupings rather than necessarily exclusive categories.

The simplest criterion is the shape of the term placenta, but potentially more relevant to physiological exchange is the histological structure and organization of the layers separating fetal and maternal blood circulations at the fetomaternal interface (Grosser classification, Amoroso, 1952; Steven, 1975a). The structure of areas outside or bordering the main placenta (accessory or paraplacental regions) and the amount of maternal tissue shed at parturition are also characteristic. These criteria all refer to the structure at term but the developmental history of the placental membranes may also provide important clues to functional relationships.

(b) Classification by shape

The fetomaternal exchange area is enormously increased by elaboration of villi or folds. The distribution of the chorionic villi is characteristic among families and has four main categories (Figure 4.4).

1. Diffuse: villi over entire surface, e.g. pig, horse.

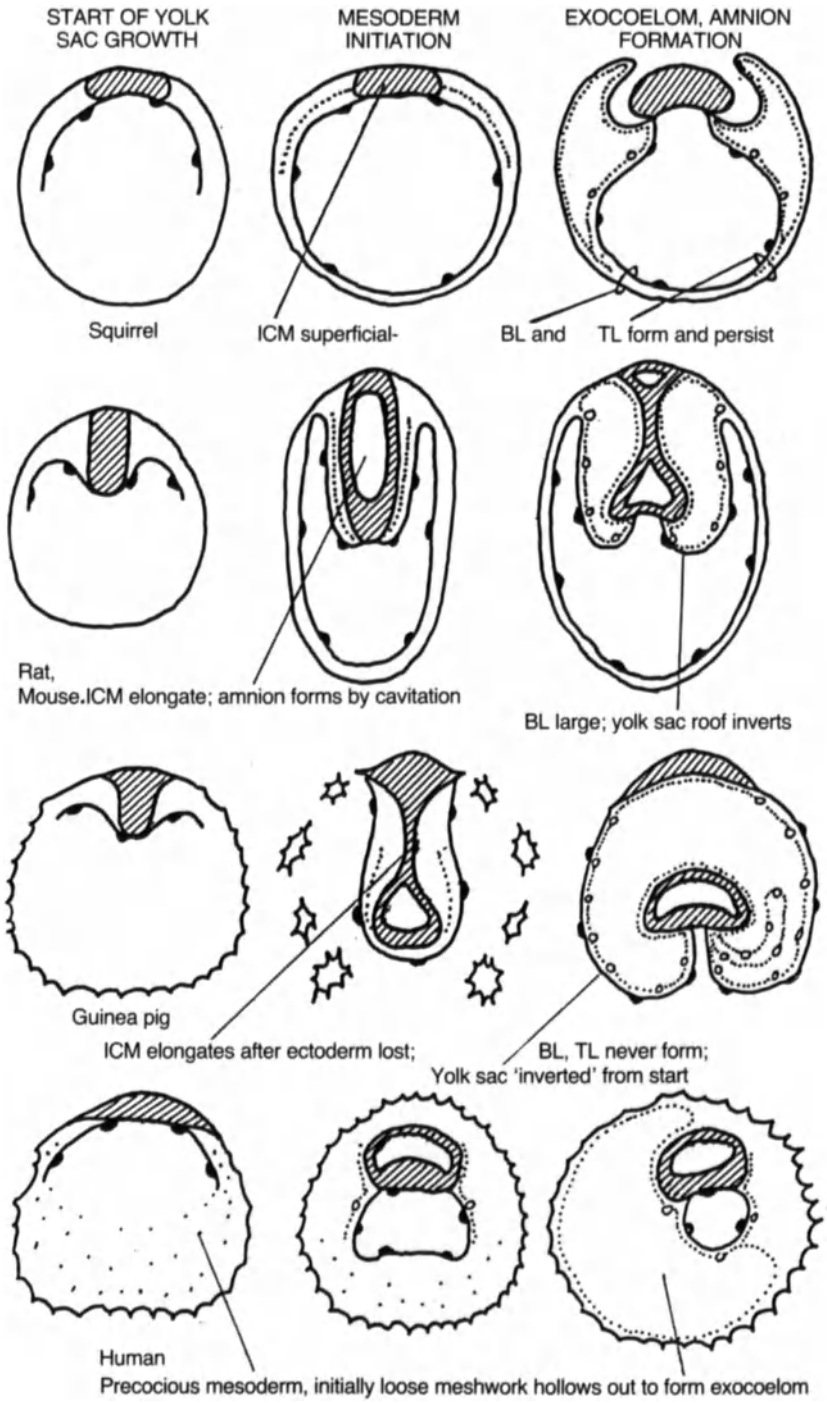
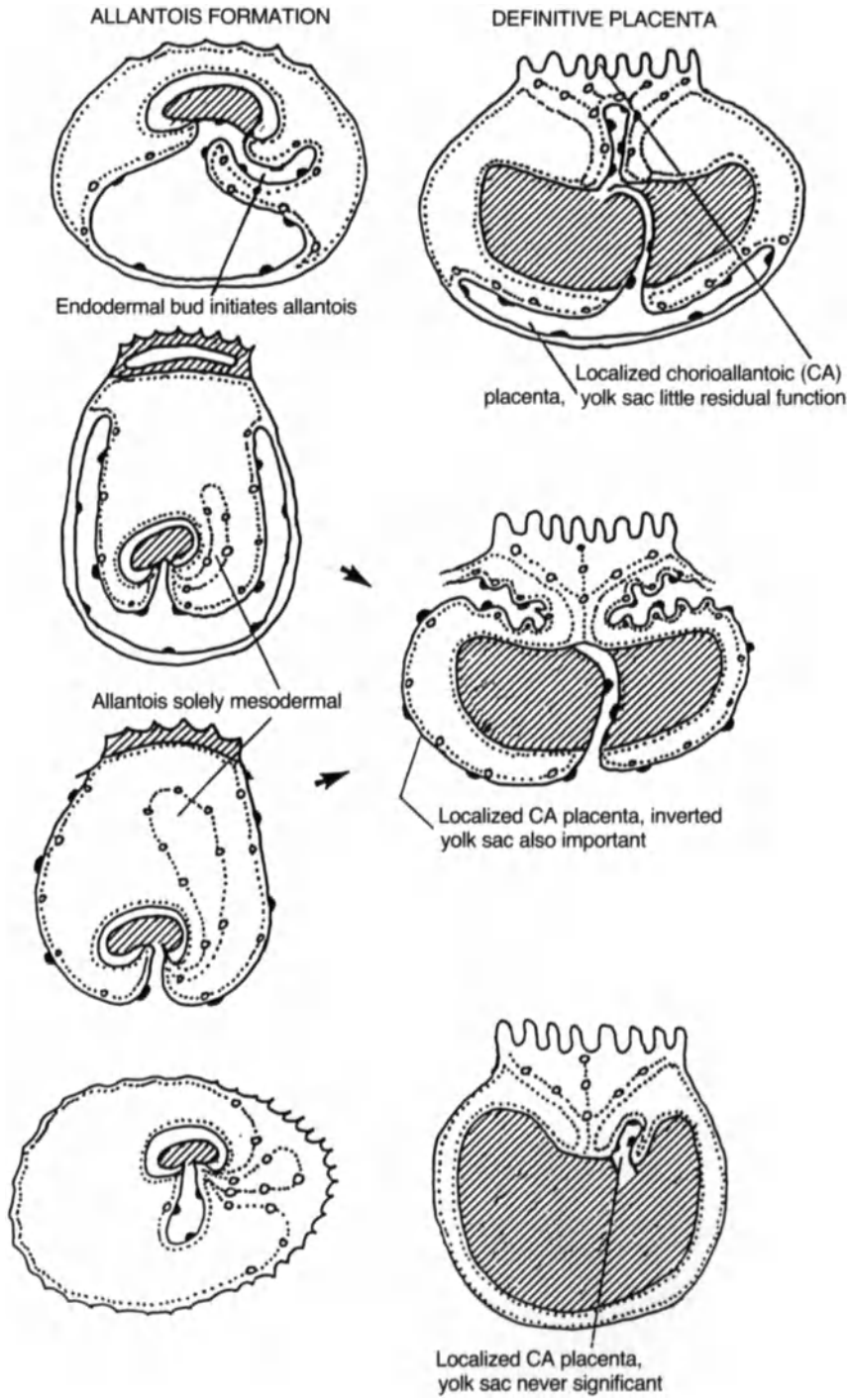


Figure 4.2 Development of placental membranes in squirrels, rat and mouse, guinea pig and human. For identification of individual membranes see Figure 4.1.


ALLANTOIS FORMATION
DEFINITIVE PLACENTA
Endodermal bud initiates allantois
Localized chorioallantoic (CA)
placenta, yolk sac little residual function
Allantois solely mesodermal
Localized CA placenta, inverted
yolk sac also important
Localized CA placenta,
yolk sac never significant

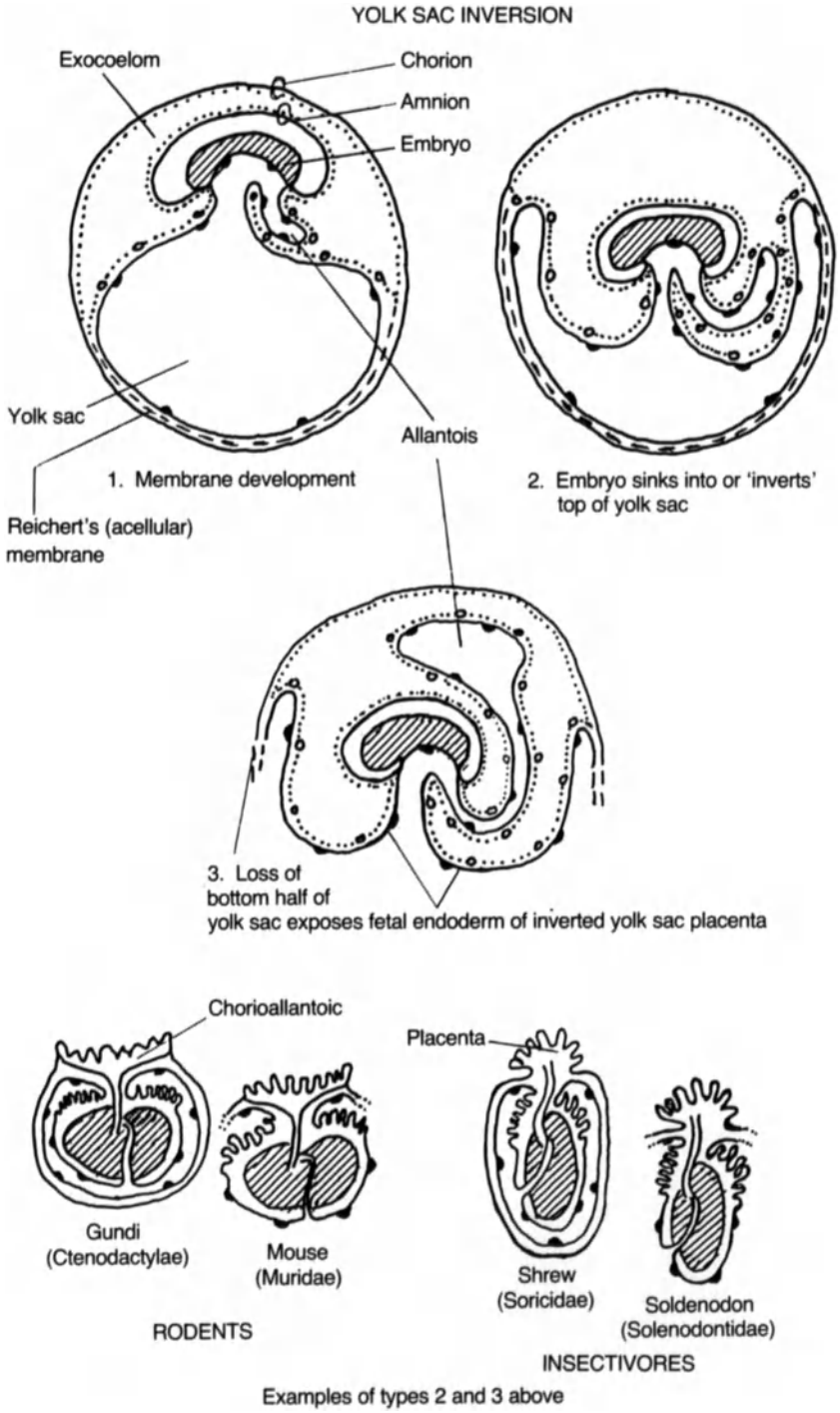


Figure 4.3 Development of the inverted yolk sac, with examples of definitive yolk sac placentation in rodents and insectivores.

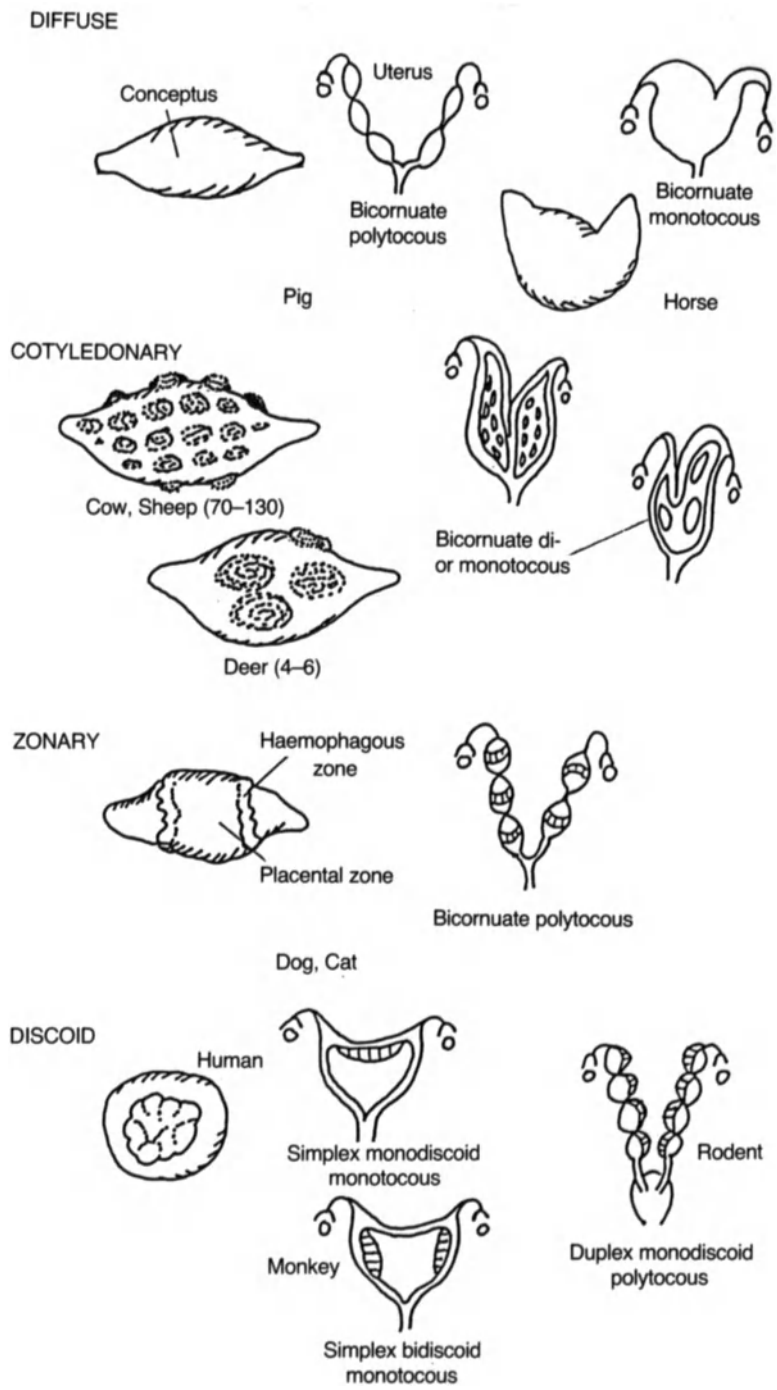


Figure 4.4 Schematic placental classification based on external form of the placenta and uterus.

2. Cotyledonary: chorionic villi grouped into a characteristic number of discrete tufts which can vary from 5 (deer) to 150 (giraffe), e.g. ruminants.
3. Zonary: villi restricted to an equatorial band or patch, e.g. carnivores.
4. Discoid: villi restricted to a single or double disc, e.g. rodents, insectivores, anthropoids.

This provides a useful simplification but within orders there are invariably exceptions outside the 'usual' category. Most insectivores have a discoid, but the American mole has a diffuse placenta; most of the ruminant placentas are cotyledonary, but that of the musk deer is diffuse; carnivore placentas vary from the usual zonary to rare discoid (brown bear) and non-carnivores are found with a zonary placenta (hyrax, dugong); a few placentas start diffuse and are discoid when mature (man, hyrax). These facts indicate that the four categories are convenient rather than functionally distinct.

Placentas with villi concentrated in limited areas (compact placentas) do have a 5–10 times greater fetomaternal exchange surface per gram weight of fetus at term than diffuse placentas (Baur, 1977), but whether this represents a higher transfer efficiency or lower safety margin in diffuse placentas remains to be established.

The uterine shape and number of conceptuses are also characteristics of a species. The uterus is based embryologically on (partial) fusion of the two Mullerian ducts to form a Y-shaped structure (Figure 4.4). The degree of fusion varies. Rats and rabbits have completely separate 'cornua' or 'horns' and this is defined as a 'duplex' uterus. Ruminants have partial fusion into common uterine body ('bicornuate'). In man and monkeys the horns are very small and the uterine body forms a 'simplex' uterus. Species producing a single offspring are designated monotocous, two ditocous, many polytocous (see Figure 4.4).

(c) Classification by histological structure

In 1909 Grosser pointed out that mammalian placentas could be grouped according to how many layers of maternal tissue were removed by the persistent chorion during development (cited in Amoroso, 1952) (Figure 4.5).

The number of tissue layers between fetal and maternal blood stabilizes after implantation and early differentiation of organ systems, and would directly affect the physiological exchange between the circulations. Grosser classified the placentas by defining first the maternal layer which was in apposition to the chorion in the definitive placenta, producing four categories (Figure 4.5):

1. Epitheliochorial: No layers removed; uterine epithelium in contact with the chorion, e.g. pig, horse.
2. Syndesmochorial: uterine epithelium removed, maternal connective tissue in contact with the chorion, e.g. ruminants.
3. Endotheliochorial: maternal uterine epithelium and connective tissue removed, maternal endothelial basement membrane in contact with the chorion, e.g. carnivores.
4. Haemochorial: all maternal tissue layers removed, chorion bathed directly in circulating maternal blood, e.g. rodents, insectivores, anthropoids.

Mossman (1937) and Owers (1960) added further categories in which the chorion was lost either from an endotheliochorial placenta (producing an endothelioendothelial) or from the haemochorial placenta (producing a 'hemoendothelial'). However, electron microscope investigations have since established unequivocally that in all chorioallantoic placentas so far examined the chorion persists to term even though it may be so thin as to be invisible at the light microscope level. The suggestion of 'vasochorial' for an endotheliochorial placenta with an especially thick interstitial membrane (Wislocki and

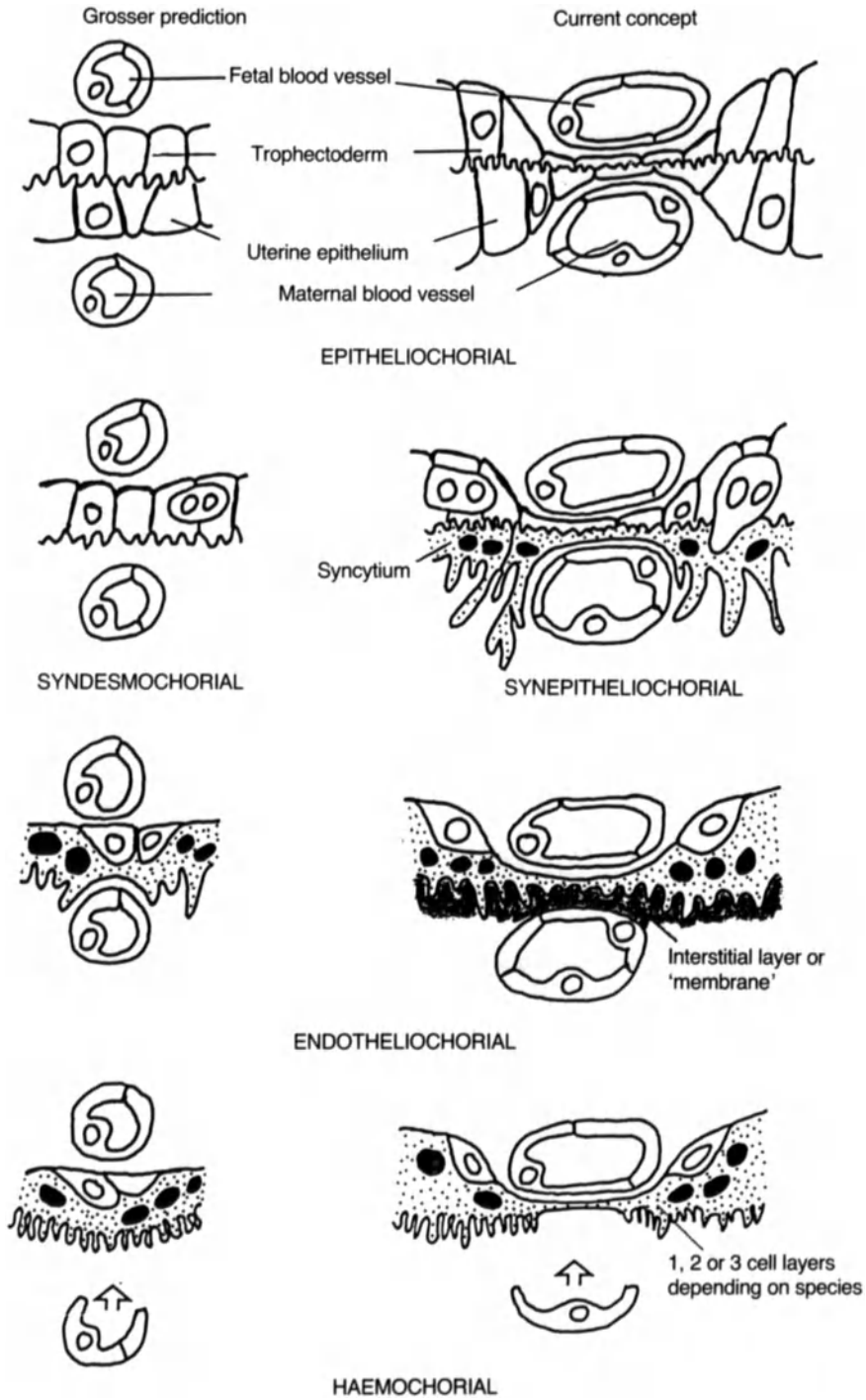


Figure 4.5 Placental classification suggested by Grosser on the left, revised to take account of recent research on the right.

Amoroso, 1956) seems an unnecessary complication of obscure derivation.

(d) Updating Grosser

Grosser initially suggested (see Amoroso, 1952) that the epitheliochorial to haemochorial series represented an evolutionary, anatomical and functional progression and proposed that, as the number of layers in what was regarded as a homogeneous semipermeable membrane decreased, the permeability and efficiency of the placenta increased. Subsequent work on capillary position, regional specialization and ultrastructure has shown that this is a considerable oversimplification (Figure 4.5).

Capillary position

By differential growth during development fetal capillaries sink into the chorionic epithelium. It is as if the capillary net expanded and pushed hard against a soft and yielding cellular layer. The capillaries always remain outside the basement membrane of the epithelium so that they are not anatomically 'intraepithelial' as usually described. They do, however, come much closer to the maternal blood, and in epithelio- and syndesmochorial placentas the maternal capillaries often indent the uterine epithelium [the analogy here would be tight string (the capillaries) around a soft parcel (the fetal villus)]. The extent to which the indentation occurs varies both within and between placental categories but generally increases toward term. There is no electron microscopic evidence that the process ever reduces the number of tissue membrane layers but the diffusion distance between the fetal and maternal circulations can be considerably reduced. This may explain why although the sodium ion permeability follows the Grosser prediction with the haemochorial (few layered) much higher than the epitheliochorial (multilayered) placentas, the gas-diffusing capacities of the two types of placenta are not significantly different (Moll, 1985). The sodium ion requires a transport system across each membrane, whereas gas diffusion depends solely on path length and concentration gradient.

Regional specialization

Biochemical, histochemical and fine structural studies have all combined to demonstrate that the placental layers can have very different characteristics in different areas of the same placenta or in equivalent layers within the same placental category. The placenta has been shown to be a very active organ metabolically and contains a wide range of selective transport systems and thus cannot be considered a homogeneous semipermeable membrane (Munro, 1986; Mossman, 1987).

Validity of the categories: EM evidence

Syndesmochorial/synepitheliochorial

Grosser defined the mature syndesmochorial placenta as having lost the maternal uterine epithelium, thereby bringing the fetal chorion into contact with the maternal endometrial connective tissue. This was based on light microscope studies which indicated that the characteristic chorionic binucleate cell of ruminants was directly involved in erosion of the uterine epithelium. Subsequent electron microscope studies could not confirm this but did demonstrate that a microvillar junction was present in all ruminants investigated, with either a syncytium (sheep, goat) or a cellular epithelium (cow, deer) on its maternal side interdigitating with the fetal chorion. This was taken as evidence that the uterine epithelium persisted (Ludwig, 1962), albeit in syncytial form in some species, and that the syndesmochorial category was therefore more accurately described as epitheliochorial with the microvillar junction being also the fetomaternal junction.

However, Wooding (1982a,b, 1984) and

C.S. Lee *et al.* (1985) have demonstrated that the sheep and goat syncytia are formed largely from the fetal chorionic binucleate cells which migrate across the microvillar junction to fuse into and form the syncytium. The layer in contact with the uterine connective tissue is therefore a fetal chorionic derivative and the placenta is legitimately described as syndesmochorial. At implantation, however, the syncytium originates from the fusion of a fetal chorionic binucleate cell with a uterine epithelial cell and the relative contributions of fetal and maternal tissue to the mature sheep or goat syncytium is still uncertain.

In cow and deer a fetomaternal syncytium is formed in the same way at implantation, but is subsequently displaced by regrowth of the residual cellular uterine epithelium. Continued binucleate cell migration across the microvillar junction produces fetomaternal trinucleate cells throughout pregnancy. These minisyncytia do not spread further but die and are resorbed after releasing their fetally produced granules to the maternal connective tissue (Wooding, 1982a,b).

All ruminants so far studied have binucleate cells and/or mini- or more extensive syncytial formations throughout pregnancy. Production of fetomaternal hybrid tissue in contact with the maternal connective tissue thus appears to be characteristic of ruminants. It is so unlike anything that is found in the other placental categories that it seems useful to maintain the distinction between epitheliochorial and syndesmochorial placentas.

The prefix 'syndesmo' was used by Grosser as a rather obscure synonym for connective tissue (*Borland's Medical Dictionary*: syndesmo, a continuing form denoting a relationship to connective tissue). It is now clear that the uterine epithelium is not removed by the chorion but more or less extensively modified by fusion with binucleate cells from implantation to term. The chorion is thus in contact with a fetomaternal syncytium and not with connective tissue. A usefully descriptive term for this situation which retains a reference to the older usage would be 'synepitheliochorial' (Wooding, 1992) (section 4.3.6b).

Haemochorial

Enders (1965), in an elegant comparative study, demonstrated that the haemochorial placenta does not always have a single layer of chorion. Different species show a constant pattern of one, two or even three separate layers between maternal blood and fetal endothelium. The terms haemomonochorial, -dichorial and -trichorial were introduced to accommodate this (Figure 4.76, section 4.3.6d). Further complexity was evident in that any layer could be either cellular or syncytial and some layers had macroscopic pores through their entire thickness. Most layers were thin, but this varied considerably. Thus, again the Grosser category covered a much wider range of structure than originally realized. There is an equivalent variation in functional capacity; the gas-exchange values for haemochorial placentas cover such a range that they include the values for epitheliochorial placentas (Moll, 1985).

Although many of the initial assumptions and predictions of the functional significance of the Grosser classification have been shown to be incorrect, the basic categories are still easily recognisable and nothing more convenient has yet been found to replace it (Figure 4.5).

Grosser also separated placentas into villous or labyrinthine types depending on the way in which the continuous increase in the fetomaternal junctional area throughout pregnancy was organized. In villous placentas the chorion initially forms simple finger-like projections which subsequently may become profusely branched but remain separate. Conventionally 'primary' villi are formed by a solid mass of ectoderm. These are said to

be 'secondary' villi when mesoderm grows into their core and 'tertiary' villi when this core vascularizes (Steven, 1975a).

In labyrinthine placentas the fetomaternal junction forms a network. This can be produced by fusion between the branches of an initially villous placenta, by a hollowing out of spaces in solid trophoblast or by the engulfment of a meshwork of maternal capillaries by the invasive chorion which replaces the maternal connective tissue. Although the structural development is usually villous to labyrinthine there are instances, such as man, where the initially labyrinthine placenta becomes villous secondarily.

(e) Classification by tissue lost at parturition

In the 'deciduate' type (see section 4.2.8) a variable amount of maternal tissue is lost with the fetal membranes at birth. The non-deciduate (or adeciduate) placenta separates at the fetomaternal junction. There is a reasonable correlation with the Grosser classification in that if there is any erosion of the maternal layers during placental development then the placenta is shed together with maternal tissue at term, which is defined as decidua. Since the data are relatively easy to collect, this classification can serve as an initial indication of structure. In contradeciduate placentas some fetal tissue is retained and resorbed after parturition. This has been reported in only a very few epitheliochorial placentas (European mole, lemurs; Amoroso, 1952).

It was originally thought that the specialized tissue (decidua) formed early in pregnancy from stromal cells in the endometrium of species with haemochorial placentation was related to the eventual placental separation zone. However, the extent of its production is variable, it frequently disappears before parturition and is completely absent in 'deciduate' endotheliochorial placentas. It therefore seems very unlikely that decidual tissue only plays a major role at parturition. For a more detailed discussion of decidua see section 4.2.8.

(f) Classification by accessory placental structures

These do not form a comprehensive scheme but are consistent features and provide a basis for further characterizing categories in the other schemes such as Grosser's. These structures are normally outside or at the edge of the main placental region and may be very different in structure.

Haemophagous zones/organs

These structures are also known as haematomas, but this term is already used in other less specific contexts and is best avoided; they occur in many endotheliochorial and some epitheliochorial placentas, normally around the margin of the main placenta but occasionally centrally (see review by Ulysses *et al.*, 1972, and Burton, 1982).

The haemophagous zone is a specialized area where the chorion and maternal tissue are widely separated by stagnant, but not necessarily clotted, maternal blood. There is no circulation of blood, the supply is by intermittent leakage and the control not understood. The chorionic trophoblast cells are typically very tall and columnar and full of large vacuoles containing phagocytosed red blood cells in various stages of digestion. It is assumed that by breakdown of maternal haemoglobin these zones provide a principal source of fetal iron.

Haemophagous zones are characteristically found at the edge or centre of the zonary endotheliochorial labyrinthine placentas of carnivores, but also occur in some endotheliochorial placentas in insectivores and bats. Some synepitheliochorial placentas also show haemophagous zones at the base of the fetal villi in the cotyledons (sheep and goat), but none has been reported in epitheliochor-

ial and only two in haemochorial placentas (shrew, B.F. King *et al.*, 1978; tenrec, Mossman, 1987).

Areolae

Areolae consist of small areas of the chorion overlying the mouths of the uterine glands whose secretion separates the two epithelia. The chorionic surface is normally elaborated in small folds carrying tall columnar cells showing evidence of active phagocytotic uptake of uterine secretions (Figures 4.20, 4.21 and 4.35). There is evidence of both merocrine and holocrine secretory modes from the uterine glands and epithelium. In the pig there is ultrastructural evidence for secretion of the iron transport protein uteroferrin from the glands and uptake by the chorionic areolar epithelium (Raub *et al.*, 1985). Enzymes related to sodium transport processes have also been localized to the areolar chorion (Firth *et al.*, 1986a,b).

Areolae are characteristic of epitheliochorial placentas and occur in large numbers over the entire surface of the placentas of pigs and horses (8000–9000 in pig, Amoroso, 1952). They are also common in the intercotyledonary areas of synepitheliochorial placentas (ruminants). In both groups large amounts of uterine gland secretion (also known as uterine milk) are produced and the areolae represent a localization and concentration of the uptake function common to all chorions. Their prominence in epithelio- and synepitheliochorial placentas may be correlated with the relative impermeability of such multilayer structures to non-diffusible substances; they are much simpler and less frequent in paraplacental regions in the cat and dog (endotheliochorial) placentas.

Chorionic vesicles

These may be derivatives of the chorionic roof of an areola invaginated into the allantoic sac (Figure 4.21). They range from vesicles lined with well-preserved chorionic epithelium containing material identical to that present between the epithelia in an areola, to sacs with no discernible internal structure (Amoroso, 1952; Mossman, 1987). These possibly represent the initial and final stages of such structures. They occur in placentas with areolae but are so infrequent and sporadic in their distribution that it seems unlikely that they have any specific function other than removal of excess secretion from between the fetomaternal layers. This is corroborated by the observation that in the horse the remnants of the endometrial cup (see below) are removed from the uterine surface and stored in a very similar structure (Figure 4.26b).

Chorionic girdle and endometrial cups

In equids before implantation, a girdle of binucleate cells develops in the trophectoderm surrounding the conceptus (Figures 4.26 to 4.30). These fetal cells migrate across the uterine epithelium to form a ring of solid structures in the uterine subepithelial connective tissue known as endometrial cups (Figures 4.26 and 4.32). These aggregates of fetal cells produce large amounts of equine chorionic gonadotrophin (eCG), previously known as pregnant mare serum gonadotrophin (PMSG), during the second quarter of pregnancy. The cells subsequently are killed by maternal leucocytes and the remnants are encapsulated by the chorion and invaginated into the allantoic cavity. This remarkable system (Figures 4.26a and b) is found only in the Equidae, but the binucleate nature, migration and hormone production of these cells are very reminiscent of the binucleate cells in the ruminants. (For further details and references, see section 4.3.6a.)

Yolk sac structures

As discussed above, the yolk sac membranes display a wide range of structural organization throughout gestation in eutherian

mammals. In the present context it is the usually transient presence of the choriovitelline placenta and the structure and position of the yolk sac at term which are used as diagnostic features.

The choriovitelline placenta (vascularized trilaminar yolk sac apposed to uterine tissue) forms the definitive placental structure in metatheria (marsupials) and is temporarily present in some families of nearly all eutherian orders whatever their eventual chorioallantoic placental type. It is important at implantation in subsequently endotheliochorial carnivores but much more transient in ungulates. In both, the vascularized yolk sac is soon separated from the chorion by expanding exocoelom, and by mid-gestation it is regressing in size. It soon becomes vestigial in ungulates but may retain some (unknown) function in carnivores and some bats (Figures 4.1 and 4.2).

In the largely haemochorial rodents, insectivores and lagomorphs the choriovitelline placenta may be present during development, but these orders are characterized from an early stage until term by the presence of an inverted yolk sac placenta which may be complete or incomplete (Figure 4.3). The incompletely inverted type has many layers but is still reported to be active in absorption in some sciurid rodents (squirrels) (Mossman, 1987). The completely inverted type has fewer layers and several studies have demonstrated its active role in macromolecular uptake including specific transport of immunoglobulins, in mouse, rat and guinea pig. Typically the relationship with the maternal tissues is far less intimate than in the choriovitelline placenta, the yolk sac tissue absorbing secretions like a fetal areolar epithelium by endocytosis from the uterine lumen (Bainter, 1986).

Subplacenta

Unique to hystricomorph rodents, a subplacenta is found on the maternal side in the central area of the placenta (Davies *et al.*, 1961a,b) (Figure 4.6 and 4.72).

It consists of trophoblast villi lacking a fetal blood supply which penetrate into the decidualised area of what becomes the placental stalk. The villi are covered by cytotrophoblast underlying a syncytial trophoblast layer of characteristic ultrastructure which proliferates to form closed lacunae not perfused by maternal blood (Figures 4.6 and 4.7).

This absence of circulating blood and the ultrastructure make the subplacental syncytium quite unlike that in the rest of the placenta. A secretory rather than absorptive function has been suggested (Davies *et al.*, 1961a,b).

4.2.4 DEFINITIVE PLACENTATION

The 'mature' or 'definitive' placenta may be characterized as the one whose structure shows no significant differences in number or type of layers between fetal and maternal blood when compared with the term placenta. If two types coexist (synepitheliochorial and epitheliochorial in sheep; haemochorial and inverted yolk sac in rodents) then usually one of them has a considerably larger fetomaternal exchange area and is quoted as the definitive form.

Often a functional yolk sac precedes the development of the chorioallantoic placenta. The growth of the fetus also has two phases: an initial implantation and differentiation of the organ systems and a subsequent exponential growth phase. A prerequisite for this rapid growth is a vast increase in the placental capacity for nutritive and gas exchange. It is to meet this demand that the chorioallantoic placenta has attained its characteristic or definitive structure usually by the second half of pregnancy and frequently much earlier, but always by the time that the maximum rate of fetal growth occurs. Baur (1977) has shown for a variety of placental structures that the absolute area of the fetomaternal junction increases continuously

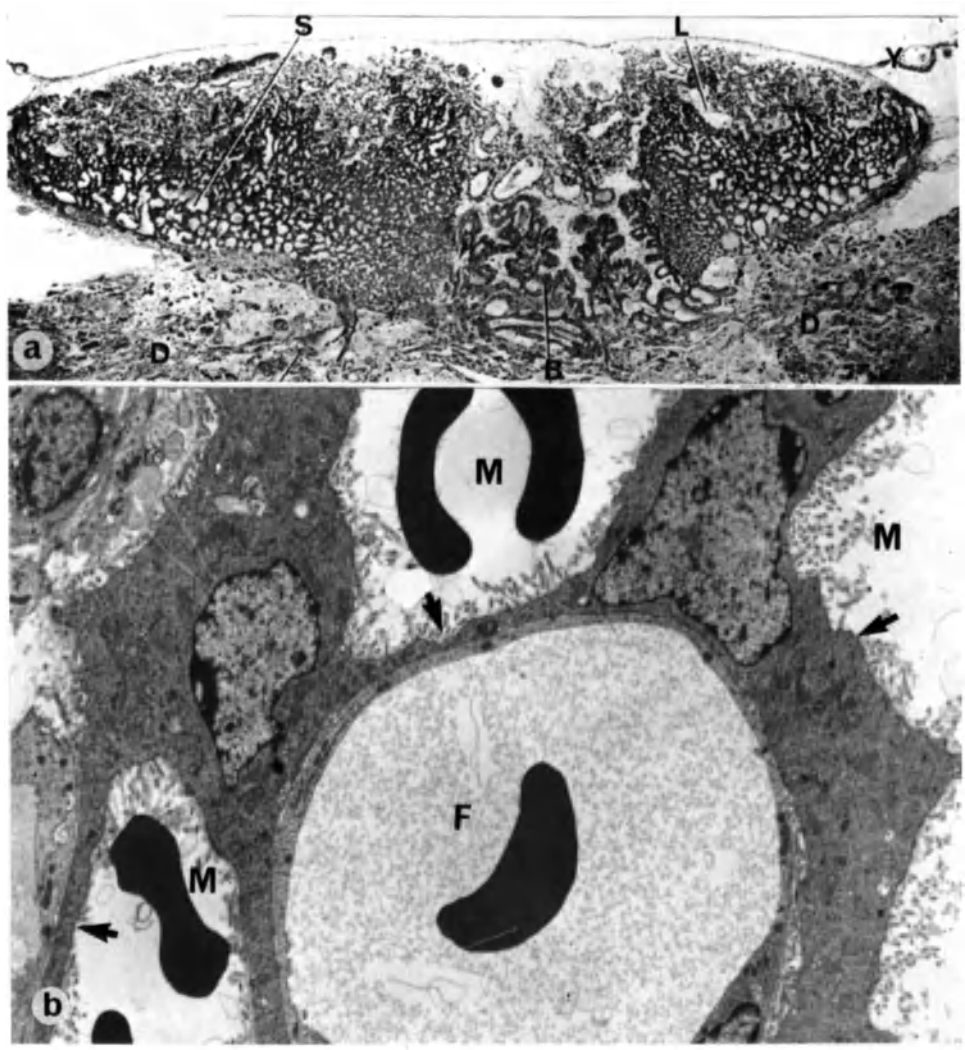


Figure 4.6 Haemomonochorial guinea pig placenta. (a) Light micrograph of a transverse section through the whole placental disc illustrating the different regions of the placenta. B, subplacenta; D, decidua; L, labyrinth; S, spongiotrophoblast; Y (inverted), yolk sac. (b) Electron micrograph showing the labyrinth structure. Note the variation in thickness (arrows) of the single syncytial trophectodermal layer. F, fetal capillary; M, maternal blood spaces. (a) 23 dpc, × 20. (b) 39 dpc, × 5200.

throughout pregnancy although the area per unit weight of fetus usually decreases significantly just before term. This indicates that the existing area of the placenta needs to be used with ever-increasing efficiency, and in most placentas the layers between the fetal and maternal circulations do get progressively thinner, which would be one way of achieving this. The fetal and maternal capillaries approach more closely by indentation of the layers and may also increase in width and frequency. The type and number of layers remain, however, unchanged, the modification being merely a refinement of a pre-existing structure (Figure 4.25).

It has been suggested that, ontogenetically,

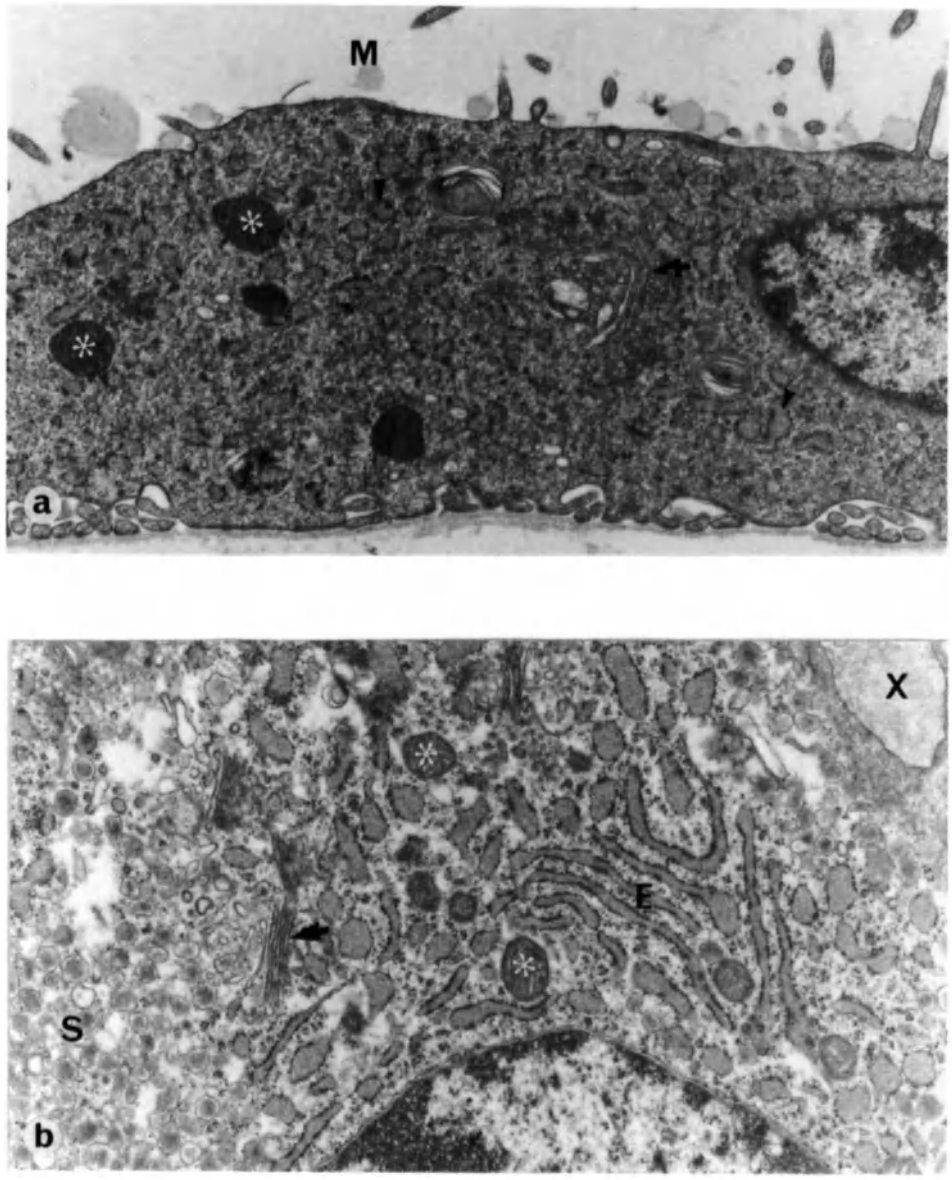


Figure 4.7 Haemomonochorial guinea pig placenta. (a) The spongiotrophoblast fine structure is very similar to, and continuous with, the trophectodermal syncytium of the labyrinth. Note the large mitochondria (asterisks), vesicular rough endoplasmic reticulum (arrowheads) and Golgi body (arrow); M, maternal blood space. (b) The ultrastructure of the subplacental syncytium is markedly different from other placental syncytium. It has arrays of endoplasmic reticulum (E) and numerous characteristic small secretory granules (S) produced from the Golgi body (arrow). X, intrasyncytial lacuna which usually is edged with microvilli. (a) 39 dpc, × 13 400. (b) 39 dpc, × 14 000.

haemochorial placentation is developed via epitheliochorial and endotheliochorial stages (Amoroso, 1952). However, this is only approximately true even at the invading front of the (usually syncytial) trophoblast because of the speed and lack of any synchrony in the development. There is no stage at which there is a significant area of (for instance) endotheliochorial placental structure in human or rodent haemochorial development. Behind the invading 'front' the definitive placental structure is immediately established and apart from 'fine tuning' in respect of diffusion distances no change in basic or definitive structure subsequently occurs, merely an increase in area. This again emphasizes that the different placental types are equally successful solutions to a particular cluster of environmental pressures. If one type of chorioallantoic placenta was more efficient, as the demands of the fetus increased it might be expected that this type would predominate since the placental area is growing continuously after establishment until term and thus is susceptible to continuous modification.

The definitive placental structures may thus be defined as those found in the main placenta close to term. Accessory placental structures should also be considered although they do not normally constitute a significant proportion of the total area. In the bovine cotyledonary placenta for example the intercotyledonary area represents less than 2% of the total area available for exchange at term (Baur, 1972) but it may have an essential functional role.

4.2.5 MATERNOFETAL EXCHANGE

(a) Histotroph and haemotroph

Conventionally there are two sources of fetal nutrition: histotroph, extracellular material including cellular debris secreted into the space between maternal and fetal surfaces in direct contact with the fetal trophectoderm, and haemotroph, materials carried in the maternal blood.

Histotroph

Since maternal blood can supply all of the needs of the mother and those fetuses with haemochorial placentation, the persistence of histotroph presumably reflects the inability of nonhaemochorial placentas to extract and transfer sufficient materials from maternal blood at a lower energy cost than absorbing a specific secretion. Phagocytosis of histotroph is considered to be a characteristic of both cellular and syncytial trophoblast. Uptake by this means is usually concentrated in specific regions such as areolae (pig, ruminants) or haemophagous zones (carnivores, sheep).

Haemotroph

Haemotrophic nutrients cross the interhaemal barrier by three major mechanisms: diffusion, endocytosis and the carrier-based systems facilitated diffusion and active transport (for a good general review of transport mechanisms, see Schaerer *et al.*, 1991). Small lipid-soluble molecules (such as oxygen, carbon dioxide and urea) cross by diffusion, a process involving no specificity of transport and only occurring down a concentration gradient. There are other devices, such as the differential affinities of fetal and maternal haemoglobin for oxygen, which serve to maximize the diffusion gradient.

Although increasing numbers of reports are appearing categorizing regional structural differentiation of the placental membrane (Ludwig, 1968; Enders, 1982; P. Kaufmann 1982), as yet only the simplest correlations of structure and function are possible. Reduction of the diffusion path length to facilitate respiratory exchange and modifications of cell structure to enhance endocytotic absorption are the two most easily recognized systems. Respiratory specializ-

ation manifests itself by attenuation of, and indentation by capillaries into, the respective fetal and maternal (if present) epithelia to bring both bloodstreams as close as possible. Absorptive specialization is recognized by membrane elaborations (microvilli, small folds), the presence of numerous coated pits, extensive apical tubulovesicular, endosomal and golgi systems plus a large lysosomal compartment. Tight junctions are also necessary in cellular epithelia to prevent passive return flow. Recent *in vitro* studies with tissue culture or isolated cells indicate that most fluid and macromolecular cellular uptake is via the coated vesicle system rather than macro or micropinocytosis (Rodewald and Kraehenbuhl, 1984; Willingham and Pastan, 1984). It occurs by non-specific fluid phase uptake (endocytosis) into the lumen of the coated vesicle and specific receptor-mediated binding by molecules in the same coated vesicle membrane. All material is then internalized via a receptosome/endosome compartment to the apical tubulovesicular system, which in some cells may be closely related to the Golgi body. Here it is sorted and dispatched either to lysosomes in coated vesicles for degradation, or to the basolateral cell membranes in smooth vesicles for release unchanged. The total fluid uptake can be enormous – up to the entire volume of a fibroblast or macrophage in 6–12 h (Steinman *et al.*, 1983). The few placental systems investigated so far with ultrastructural tracer techniques indicate the general validity of the model in this context (bat: Enders and Wimsatt, 1971; guinea pig: King and Enders, 1971; Moxon *et al.*, 1976; Kimberley and Thornburg, 1989).

Hydrophilic solutes with a low permeability coefficient in lipid membranes (such as glucose) are frequently transported by facilitated diffusion, that is by a process involving a membrane-bound carrier molecule which displays a substrate specificity and can be saturated, but again only down a concentration gradient. Where energy is utilized to drive the process of facilitated diffusion against a concentration or charge gradient, the mechanism is named active transport; amino acids are transported in this way. Here the relevant carrier molecule is located in the plasmalemma and has a receptor and transfer function. Usually the import of one molecule such as an amino acid is coupled to the export of another (in this case sodium ions). The imported molecule then has to utilize a similar system to cross the basolateral plasmalemma before it can pass right across the epithelium. Breakdown products from the lysosome may also cross lysosomal and cellular membranes by carrier systems. There is no good ultrastructural evidence for release of lysosomal content by exocytosis. Morphological evidence for carrier-mediated transport can only be obtained if the carrier protein can be localized by histochemical or immunocytochemical methods. So far for the placenta this has been limited to broad categories of enzymes – acid or alkaline phosphatases for example – the precise roles of which are unknown. However, recent work on Na/K-ATPase (Skolek-Winnisch *et al.*, 1985; Firth *et al.*, 1986a,b) and the steroid interconversion enzymes (Farkash *et al.* 1986) does show the potential of the technique. Carrier-mediated transport will be enhanced by short diffusion path lengths between membranes given an equal number of membrane barriers to cross. Thus, the capillary indentation and attenuation of the fetomaternal interface epithelia will facilitate this type of transport as well as respiratory exchange.

The structural criteria described for the various fetomaternal transfer systems can be used to identify potential regional specializations in many different types of placenta. Evidence that such areas function as proposed requires detailed tracer and physiological studies.

(b) Transport processes: quantitative aspects

Clearly all of the substances required for the growth and metabolism of the fetus, including water, oxygen, carbohydrates, amino acids, non-esterified fatty acids, metal ions and trace elements enter the fetal from the maternal circulation. Products of metabolism such as carbon dioxide and urea, as well as certain other compounds, flow in the opposite direction. Placental intervascular transport (either chorioallantoic or vitelline, or both) is the principal route of fetomaternal exchange. (See Morriss and Boyd, 1988, and Sibley and Boyd, 1988, for recent comprehensive reviews.)

Some indication of the quantities of material transported can be obtained from the following: in the fetal lamb late in gestation 7.8 g of carbon/day per kg fetal weight enters the fetal circulation, of which 4.6 g/day per kg is returned as carbon dioxide and urea (Battaglia and Meschia, 1978). Estimates of amino acid uptake made in fetal lambs between 118 and 146 days' gestation show that approximately 11 g of amino acids enters the fetal circulation per kg fetal weight per day, of which approximately 4.5 g is accumulated as new tissue (Lemons *et al.*, 1976; Battaglia and Meschia, 1978). Oxygen consumption by the sheep fetus between 80 days' gestation and term is approximately 8 ml oxygen STP/min per kg fetal weight; the quantity of substrate oxidized by this oxygen (caloric equivalent) is 58 kcal/day/kg, and at 130 days' gestation an additional 32 kcal/day/kg is accumulated as new tissue (Rattray *et al.*, 1974; Battaglia and Meschia, 1978). The mean growth rate of lambs at 130 days' gestation is 36 g/day/kg (Rattray *et al.*, 1974). A.W. Bell (1991) provides an excellent review of the maternal metabolic adaptations to the demands of pregnancy and quantitative aspects of transport to and metabolism in the developing conceptus.

Rates at which substances enter the fetal circulation are most frequently estimated from direct measurements of umbilical arterial and venous concentrations (venoarterial differences) multiplied by umbilical blood flow (the Fick principle), and unless otherwise indicated the data described here were obtained in this way, usually in late gestation. However, this technique cannot be applied to solutes for which placental uptake or clearance represents only a small proportion of umbilical concentration, because of the difficulty of measuring small differences in concentrations between the two vessels, and it is difficult to apply in small animals because of the size of the vessels; in these cases only a more indirect estimate of transfer rates can be obtained from measurements of the differences in concentration of radioactively labelled solutes introduced into the maternal or fetal circulations. Reliable data can only be obtained in chronically catheterized animals which have been allowed sufficient time to stabilize following surgery. Data for the human fetus are predictably scarce, although umbilical arteriovenous concentration differences can be obtained at Caesarean section. Methods used have been reviewed in detail in Nathanielsz (1980), Battaglia and Meschia (1986) and Molina *et al.* (1991).

Oxygen, carbon dioxide and urea cross the placenta, as they do other epithelia, by diffusion. The rates of gaseous diffusion across the placenta depend on the diffusion distance and are independent of the number of tissue layers involved. It must be remembered that the placenta itself consumes up to 40% of the total oxygen uptake by the uterus in late pregnancy (see discussion in A.W. Bell 1991). Fetal oxygen consumption does not appear to be limited by the rate at which it diffuses across the placenta since acidbase balance studies show that under normal conditions there is no metabolic acidosis in the fetus of the sheep, calf, horse or man; furthermore raising fetal Po_2 by transiently administering 100% oxygen to the mother does not alter

fetal oxygen consumption (see Battaglia and Meschia, 1978).

However, this leaves unexplained the results in the horse, which seem exceptional in this respect (Silver *et al.*, 1973; Silver and Steven, 1975); differences between the oxygen tensions in umbilical venous and uterine venous blood (Table 4.1) show that transfer in the mare is clearly more efficient than that in sheep (Wilkening and Meschia, 1992).

Uptake of oxygen by uterine tissues, or by the interhaemal tissues constituting the barrier between maternal and fetal blood, is a minor factor contributing to this difference; the major factor has been suggested to be the countercurrent relationship between fetal and maternal microcirculations in the microcotyledons in the mare compared with the less efficient cross-current flow in the sheep (Figures 4.8 and 4.9). This is dealt with in more detail in section 4.2.5(c).

Glucose is the most important single source of fetal energy (Molina *et al.*, 1991). It is transported by facilitated diffusion (sheep, guinea pig, Folkart *et al.*, 1960; Bissonnette, 1981; man, Carstensen *et al.*, 1977; Ingermann, 1987). This is shown by the dependence of the transfer on fetal and maternal glucose concentrations, which are in turn controlled principally by glucose ingestion and circulating insulin levels. Injection of insulin into the fetus decreases fetal blood glucose concentration by increasing utilisation and increases the flux from the mother. Maternal starvation reduces the flux and lowers fetal insulin (Boyd *et al.*, 1973). However, fetal oxygen consumption does not drop as much on maternal starvation as would be predicted, on the basis of reduced glucose transport, indicating an alternative fetal energy source, probably lactate and amino acids. Lactate, which is produced in the placenta (Burd *et al.*, 1975), is transported from mother to fetus by facilitated diffusion but its use has attendant dangers since a high circulating level can damage the fetal brain. A lack of glucokinase in most fetal livers ensures that glucose is delivered preferentially to the brain and nervous system. The fetal liver can efficiently utilize a variety of other substrates including lactate and galactose (Sparks *et al.*, 1976) but fructose is not metabolized despite its rapid synthesis in and transfer from the placenta and its high concentration in the fetuses of several species (Tsoulos *et al.*, 1971).

There are only limited differences between placentas of different structures in the characteristics of amino acid transport. Concentrations of amino acids in the fetal circulation are in general higher than those in maternal blood and this, together with a good deal of other evidence, indicates that most amino acids are transported across the placenta by active transport (Battaglia and Meschia, 1978; Young, 1981, 1988; Battaglia, 1992). The transport process involves the accumulation of amino acids to high levels in placental tissue, from where they diffuse into the fetal circulation; in addition there is some placental amino acid metabolism. Since they have different carriers, groups of amino acids differ in the rates at which they are transported: branched-chain amino acids such as valine, leucine and isoleucine are readily transferred from mother to fetus in the sheep, guinea pig and monkey, but basic amino acids cross relatively slowly in the sheep (see Young, 1981, 1988; Edwards *et al.*, 1993). Acidic amino acids such as aspartate and glutamate do not cross the placenta readily, in keeping with their behaviour in other transport systems such as those of the small intestine and blood–brain barrier. On infusion of ^{14}C-labelled aspartate and glutamate into the pregnant rhesus monkey, Stegink *et al.* (1975, 1979) observed labelled catabolic products such as glucose and lactate in fetal blood, but none of the parent compounds. The fetus is, in fact, capable of the synthesis of these amino acids, and there is a concentration gradient for glutamate across the placenta from fetus to mother, indicating that glutamate may be secreted by the fetus;

in the fetal lamb this flux may reach 5 mmol/day per kg of fetus (Lemons *et al.*, 1976). The glutamate is produced by glucose/lactate catabolism in fetal liver and converted to glutamine in the placenta. This utilizes the considerable ammonia production by the placenta, which has low urea cycle activity. The newly synthesized glutamine is then passed back to the fetus for use in protein synthesis (A.W. Bell, 1991).

Estimates of amino acid uptake made by measuring the nitrogen content of the fetus at various stages of gestation indicate that the fetal lamb accumulates approximately 0.65 g of nitrogen/kg/day (Battaglia and Meschia, 1973); estimates of amino acid uptake based on umbilical concentration differences in the same species are of the order of 1.5 g of nitrogen/kg/day (Lemons *et al.*, 1976). The disparity can be accounted for by deposition and metabolism in the placenta and nitrogen loss as urea from the fetus and placenta. Arteriovenous concentration differences for urea are too small to allow a comparable calculation, but infusion of labelled urea has shown the mean placental clearance rate to be 0.36 g of nitrogen/kg/day (Gresham *et al.*, 1972). This is consistent with the high fetal to maternal concentration gradient for urea across the placenta observed in a number of species, including man and sheep (see Battaglia and Meschia, 1978), and it is thought that almost half of the amino acids transferred are oxidised by the fetus with production of urea.

There are considerable species differences with respect to placental transfer of fatty acids. In ruminants, the placenta is relatively impermeable to long-chain fatty acids (Van Duyne *et al.*, 1960); although there may be uptake of acetate by the fetus (Comline and Silver, 1976), fetal circulating concentrations of fatty acids are low, and fat represents only a small proportion of fetal body weight at term (Rattray *et al.*, 1974). A.W. Bell (1991) provides an excellent review of the maternal metabolic adaptations to the demands of pregnancy and quantitative aspects of transport to, and metabolism in, the developing conceptus. However, some fatty acids do enter the fetal circulation from the mother, for example, essential fatty acids such as linolenic and linoleic acids. These are substrates for synthesis of prostaglandins, leukotrienes and thromboxanes, which are obligatory requirements for the fetus, and they are taken up via the placenta in all species. In contrast to the ruminants, fatty acid transport is much more rapid in some other species (mare, guinea pig, rabbit, rat, rhesus monkey and man, for example) and circulating levels of fatty acids are higher; in the rabbit fetal fatty acid uptake is so avid that starvation, which leads to an increase in maternal circulating non-esterified fatty acid level, results in increased fatty acid deposition in the fetuses (Edson *et al.*, 1975; Elphick *et al.*, 1979).

Most ions (sodium, potassium, calcium, chloride, phosphate, etc.) are transported across placentas by carrier-mediated processes at each membrane barrier, although paracellular routes ('pores') allowing passive diffusion are also considered probable, for example for sodium and potassium (Sibley and Boyd, 1988; Edwards *et al.*, 1993). However, it should be remembered that such 'pores' are merely postulates to explain physiological results (for a recent review, see Stulc, 1989): they have no convincing ultrastructural correlates in the fetal and maternal placental compartments. Each placenta relies on a variety of 'pores', carriers and cytoplasmic binding proteins, but the localization and quantitative efficiency of each system is not yet known in any detail (reviews: Shennan and Boyd, 1987; Brunette, 1988; Morriss and Boyd, 1988; Sibley and Boyd, 1988). Large molecules, for example proteins and vitamin B_{12} bound to a carrier protein, are taken into the cell by pinocytosis, but this process is by no means non-selective, since the trophoblast membranes express on their surface specific receptors. These are involved in recognition and uptake of the proteins and loaded carrier

Table 4.1 Comparative physiological data (approximate means) for term or near term placentas

Species	*Placental type*	*Relationship of maternal/fetal blood microflows from the literature***	*Fetal wt at term (g)*	*Placental wt at term (g)*	*Percentage placental/ fetal wt*	*Conceptus growth rate (slope of cube root of total volume vs age, wks)*	*Relative villous surface area (cm^2/g placenta)*
Pig	Epitheliochorial	Mixed	1300	200	15	0.65	6
Horse	Epitheliochorial	Countercurrent (maternal cascade)	60 000	4000	7	0.87	4
Sheep	Synepitheliochorial	Crosscurrent	4500	350	8	1.15	–
Cow	Synepitheliochorial	Crosscurrent	40 000	5600	14	1.08	32
Cat	Endotheliochorial zonary	Crosscurrent	100	15	15	0.77	67
Rabbit	Haemodichorial compact	Countercurrent	50	4	8	1.35	–
Guinea pig	Haemomonochorial compact	Countercurrent	85	5	6	0.62	29
Primates*	Haemomonochorial compact	Mixed	3500^{m}	500^{m}	14^{m}	0.41^{m}	14^{m}

References: Dawes (1968); Crombie (1972); Silver *et al.* (1973); Samuel *et al.* (1974); Kitchen and Bunn (1975); all data for conscious animals where available.

* data for primates from man (m) or rhesus monkey (R).
** but see pages 257 and 262 and Figures 4.8, 4.9, 4.72 and 4.88.

Species	*Minimum thickness of diffusion barrier (capillary to capillary in μm†)*	*Sodium permeability (μl/sec/g placenta)*	*Iron transport mechanism*	*Blood flow rates (ml/min/kg fetus)*		*Gaseous diffusion capacity (ml/min/mm Hg/kg placenta*
				Umbilical	*Maternal uterine*	
Pig	2–10	0.002	Uteroferrin	115	300	–
Horse	5–10	–	Uteroferrin	171	330	–
Sheep	5–10	0.04	Haemophagy	179	250	7
Cow	5–10	–	Haemophagy	232	320	–
Cat	5–10	0.06	Haemophagy	–	–	–
Rabbit	2	0.6	Transferrin	–	106	29
Guinea pig	3	0.5	Transferrin	64	160	45
Primates*	4^{R}	0.6^{R}	Transferrin	208^{R}	100^{R}	10^{m}

Baur (1977); Bissonnette and Wickham (1977); Michael *et al.* (1985); Bacon *et al.* (1984); Moll (1985); Mayhew *et al.* (1984, 1986).

† data from Wooding, unpublished.

Species	*Oxygen utilization rates (mmol/day/kg)*		*Haemoglobin concentration in fetal blood (g/100 ml)*	*Approximate P_{50} of blood* (mm Hg)*		*Fetal arterial oxygen saturation (%)*
	fetus	*adult*		*fetal*	*maternal*	
Pig	–	–	12	22	33	46
Horse	450	130	11	23	25	60
Sheep	500	260	15	17	34	62
Cow	470	140	–	22	31	50
Cat	–	–	12	36	36	–
Rabbit	–	–	14	27	31	–
Guinea pig	–	–	12	19	30	59
Primates	450[R]	450	18[R]	22[m]	26[m]	60[m]

Critchley and Burton (1987). *Partial pressure of oxygen at 50% saturation of haemoglobin.

Species	*Oxygen partial pressures (mm Hg)*				
	MA	**MV**	**FV**	**FA**	**MV–FV**
Pig	92	58	38	25	20
Horse	93	52	49	33	3
Sheep	95	51	34	23	17
Cow	82	62	38	27	24
Cat	–	–	–	–	–
Rabbit	–	–	–	–	–
Guinea Pig	–	–	–	–	–
Primates	129[R]	–	33[R]	23[R]	–

[m]man; [R]rhesus monkey.

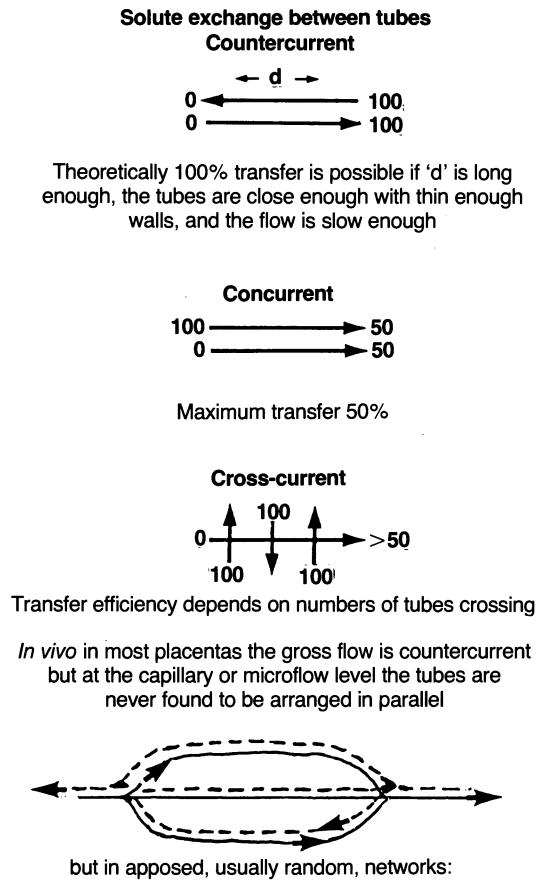


Figure 4.8 Theoretical considerations of solute exchange between capillaries.

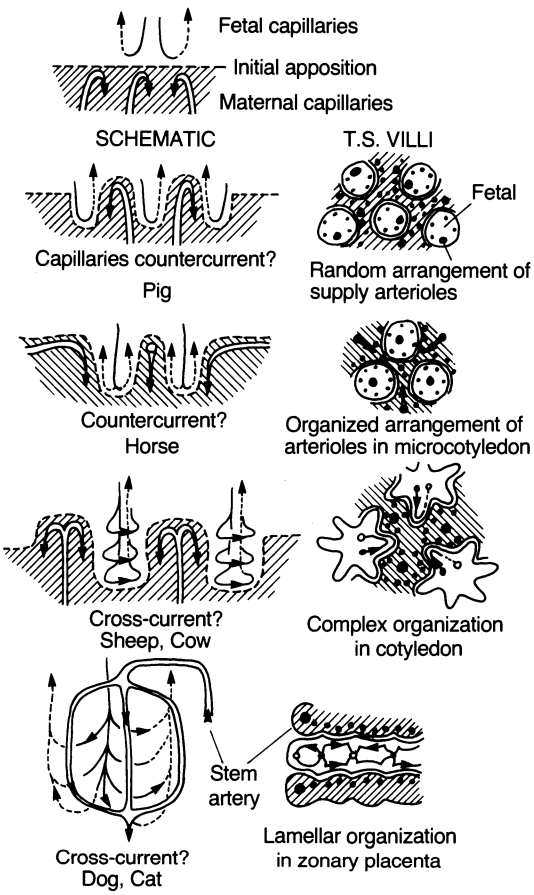


Figure 4.9 Schematic illustration of the relationship between the direction of capillary blood flows in the fetal and maternal placental circulations in various animals. See also Figures 4.8, 4.72 and 4.88.

proteins. The discussion is complicated by the fact that the transport of these molecules is not limited to one mechanism or to a single component of the placenta; the transport of large molecules can occur via the phagocytosis of uterine secretions (histotroph; see below), or of extravasated maternal blood by the trophoblast in a haemophagous zone of the placenta, or via the vitelline placenta (if present) and/or via the chorioallantoic placenta. The proportion of solutes transported

by each of these routes varies according to the type of placentation employed.

The principal evidence for pinocytotic uptake and microvesicular transfer of intact specific proteins across the placenta comes from studies with the electron microscope of the transfer of substances such as ferritin and peroxidase, and these studies emphasize the importance of the yolk sac (vitelline) placenta, particularly in the guinea pig and rabbit and to a lesser extent in rodents (Bainter, 1986). In primates this process occurs at the chorioallantoic placenta, and in animals with epitheliochorial placentas in the areolae. Other evidence for this form of transport comes from studies on the uptake of fluorescein-labelled immunoglobulin antibody proteins by the rabbit yolk sac *in vivo*. Labelled rabbit IgG introduced into the uterine lumen in 24-day pregnant rabbits was transferred across the endodermal cells of the inverted yolk sac placenta to the fetal circulation, but bovine IgG was degraded to amino acids and did not enter the fetal compartment (Hemmings, 1957). This is now known to be due to the existence of two routes for protein uptake (Bainter, 1986). Rabbit IgG is bound to specific receptors in the endocytotic (coated) vesicle membrane while bovine IgG is taken into the cell nonselectively in the fluid bulk phase within the vesicle. Proteins recognized by receptors are destined for transport to specific sites – the basolateral plasmalemma for transcellular transport or the Golgi body for modification; bulk phase proteins are transferred to the lysosomal system for breakdown. Brambell's (1966, 1970) suggestion that lysosomes are involved in selective transport of immunoglobulins has now been superseded.

Transport of specific immunoglobulins has been demonstrated in the rabbit (Moxon *et al.*, 1976; Wild, 1981) and guinea pig (B.F. King, 1977) inverted yolk sac placentas and in the primate chorioallantoic haemochorial placenta (Bainter, 1986; Leach *et al.*, 1990; Sooranna and Contractor, 1991). No such selective transport of immunoglobulins has been detected across ungulate (pig, Matre *et al.*, 1975) placentas where maternal protective immunoglobulin antibodies are transferred post-natally in the milk. There are reports of immunoglobulin transfer prenatally in carnivores (Bainter, 1986) but the site and mechanism of transport are unknown.

From the discussion above it can be seen that the rate of placental transport of nutrients to the fetus can be influenced by many factors; inadequacies result in failure of normal fetal growth (fetal growth retardation). Of most importance in relation to fetal growth retardation are the concentrations of nutrients in the maternal circulation and the transfer ability of the placenta. For those substances crossing the placenta by facilitated diffusion down a concentration gradient there will clearly be no transport if the gradient is not maintained; thus undernourished mothers provide insufficient glucose to their fetuses. The insufficiency is exacerbated by the competition for use of glucose by the undernourished mother and the placenta, when it does become available. Under these conditions active transport in the placenta is also reduced, as it receives less substrate for oxidative metabolism. Similar constraints on fetal well-being are caused by placental disease, such as pregnancy toxaemia, probably due to reduced effective placental surface area. The relationship between placental size and fetal mass is fairly direct and has been studied in sheep with reduced cotyledon numbers following uterine carunclectomy (Alexander, 1964; Robinson *et al.*, 1979). For a review of the gross macroscopic relationships between placental size and fetal mass see A.W. Bell (1984). The ways in which the fetus uses endocrine mechanisms to alter maternal circulating levels of nutrients is dealt with below under the endocrinology of placentation.

(c) Vascular relationships

General considerations

There are two placental circulations, maternal and fetal, which are separated from each other by one or more layers of placental tissue. The maternal vessels in the placenta arise from the uterine vasculature, and are closely related to those vascularizing the rest of the uterus and the ovary; in some primate species, during late pregnancy, ovarian arterial blood may also reach the placenta via uteroovarian arterial anastomoses (Wehrenberg *et al.*, 1977). As a result it is not possible to isolate major arteries supplying only the placenta; this can readily be demonstrated by infusing X-ray opaque material into the uterine artery *in vivo*, when both myometrial and placental circulations fill with tracer concurrently. The fetal side of the chorioallantoic placenta, on the other hand, receives all the blood entering it through the umbilical arteries, and is drained exclusively by the umbilical veins. Thus, although direct measurements of blood flow in the umbilical vessels give an accurate indication of blood flow in the placenta and fetal membranes, measurements of uterine arterial blood flow always include uterine and placental components. The magnitude of these flows can be judged from Table 4.1. For reviews see Dawes (1968), Rankin and McLaughlin (1979), Wallenberg (1981), Martin (1981), Silver *et al.*, (1982), Battaglia and Meschia (1986) and Mossman (1987).

The first measurements of maternal uterine blood flow were made in the 1930s by collecting venous outflow in rabbits (Barcroft, 1933); nowadays uterine arterial blood flow can be determined directly with cuff electromagnetic or ultrasonic flowmeters which, once implanted, can be used to record flows over long periods in conscious animals. Alternatively, acute experiments can be carried out using labelled microspheres to determine flows to individual tissues at the time the animal is killed. Measurements of umbilical blood flow are most frequently made using flowmeters.

For substances diffusing across the placental barrier, such as blood gases, factors influencing transfer rates include the spatial relationships between maternal and fetal blood capillaries; the fetal villous exchange area; concentration gradients across the areas of exchange; blood flow rates on either side of the barrier; the distance the gases have to diffuse between blood systems; and the rate of gas utilization or production by the placenta itself.

Probably the most important factors governing placental gaseous exchange are the vascular architecture, which determines the directions of the maternal and fetal blood flows, and the distance separating those flows. There appear to be at least four theoretical types of flow relationships: con-, cross- and countercurrent and mixtures of some or all of these together (Martin, 1981) (Figure 4.8). Of these, the countercurrent system is expected to be most efficient because blood leaving the exchange area on one side will tend to equilibrate with that entering it on the other, and a maximum solute transfer of 100% is theoretically possible. For concurrent flow such equilibration can only result in a maximum solute transfer of 50%.

It must be remembered that there are two levels to be considered, gross flow and what may be termed microflow. Gross flow is the overall direction which in all placentas so far investigated, with the possible exception of Old World monkeys and anthropoids, appears to be countercurrent. Fetal stem arteries run directly to the maternal side and maternal arteries to the fetal side before dividing up into capillaries which conduct the blood back to the veins. The microflow is at capillary level, and this is where the haemotrophic exchange takes place, most efficiently with the least practicable separation between the flows.

In all cases so far examined in sufficient

detail, the architecture of both fetal and maternal capillaries or blood spaces is a network, sometimes but not always with a predominant direction (Figure 4.8). There is no evidence for significant lengths of parallel apposed tubes of the networks which would allow sufficient equilibration of, for example, oxygen concentration as usually figured in model systems (Moll, 1972; Martin, 1981). It may be more realistic to consider that all placental capillary exchange takes place between microflows in networks which vary continuously between cross-, counter- and even concurrent and are best described as 'network current' (Figures 4.8 and 4.88).

The vascular morphology of the placental exchange region may be investigated histologically or by injection and subsequent hardening of plastic or latex followed by removal of the soft tissues and light (Figure 4.23) or scanning electron microscopic (SEM) (Figure 4.24) examination of the resulting 'corrosion casts'. Injection of visually detectable or X radio-opaque dyes (angiography) is also useful, but the latter technique is of no value at the microflow level at which exchange occurs. Of prime importance to the nature of the relationship between the gross flows of maternal and fetal blood are the positions of the arteries and veins supplying and draining the primary villi, and this can be determined by the methods described above. SEM studies of corrosion casts give exquisite detail of either maternal or fetal plumbing (Figures 4.40 and 4.47) but it is very difficult to elucidate the intimate relationship between them, since it is not yet possible to distinguish fetal from maternal capillaries unequivocally on the same preparation.

As more SEM microcorrosion cast studies are published (e.g. Leiser and Kohler 1983, 1984; Burton, 1987; Leiser, 1987; Christofferson and Nillson, 1988; Leiser and Dantzer, 1988; Ogura *et al.*, 1991), it becomes increasingly obvious that a placenta consists essentially of two bloodstreams separated by a minimum of tissue. Most conventional EM and LM sections overemphasize the tissue component. Better perfusion fixation shows that probably more than 50% of the *in vivo* placental volume is occupied by blood channels (Figure 4.44), a figure supported by measurements of the total blood volume using tracers in placentas clamped *in vivo*.

The progression (in morphological but not necessarily evolutionary terms) from epitheliochorial to haemochorial placentation occurs with increasing loss of uterine tissue, with important consequences for vascular organization (Figure 4.9). Epitheliochorial placentas are formed by a mutual growth of short fetal and maternal villi to form a structure consisting principally of two complex and extensive capillary beds. Synepitheliochorial and endotheliochorial placentas develop similarly but have much longer villi with great modification or elimination of the uterine epithelium. The maternal connective tissue remains an important constituent of maternal placental structure. Haemochorial placentas have no residual uterine epithelium blood capillaries or connective tissue, but need to deliver maternal blood up to the fetal side of the placenta from where it drains back through entirely fetal tissue. To accomplish this the main maternal arteries persist, usually passing through the full depth of the fetal villi or labyrinth (Figure 4.9). The endothelium of such arteries is usually replaced by invading fetal trophectoderm early in gestation, but the initial maternal blood conduits are retained and even greatly enlarged throughout pregnancy (Figure 4.72). The only exceptions to this are the Old World monkeys and anthropoids (section 4.3.6d and Figure 4.88). Thus, in late pregnancy there is virtually no maternal tissue left in a haemochorial placenta above the decidual layer or myometrium.

Different placental types

The following descriptions of placental vascular anatomy will be limited to the mature placentas of late pregnancy. It should be noted that placental vasculature develops throughout gestation as the fetal nutrient and blood gas requirements increase, and that total placental blood flows and chorionic villous areas increase with increasing fetal mass (Baur, 1977). In the pig's definitive placenta blunt fetal villi (length 150 μm, width 30 μm, Tsutsumi, 1962; Crombie, 1972) are inserted into maternal crypts. Each fetal villus has a peripheral meshwork of non-fenestrated capillaries usually supplied by an arteriole running up one side to the top of the villus before branching significantly into capillaries draining down to the base. The maternal crypts have a very similar arrangement, but of fenestrated capillaries, with arterial supply to the top and draining around the base (Figures 4.9, 4.23 and 4.24). The predominant capillary flow is therefore down both fetal villous and maternal crypt, in opposite directions. As can be seen from the diagram (Figure 4.9), this produces a gross countercurrent flow between the capillaries, which indent their epithelia deeply (Goldstein, 1926; Steven, 1983; Figures 4.22 and 4.25) and are presumably the main site of haemotrophic fetomaternal exchange. The corrosion cast evidence (Tsutsumi, 1962; MacDonald, 1975, 1981; Leiser and Dantzer, 1988) indicates that the microflow is between two capillary networks with no predominant direction. Microflow is therefore the mixture of cross and countercurrent, here called network flow (Figure 4.8).

In contrast to the pig, the vascular structure of the diffuse epitheliochorial placenta of the horse is more complex (Tsutsumi, 1962; Silver and Steven, 1975; Steven and Samuel, 1975) because of the formation of longer fetal villi and their organization into microcotyledons. These appear to arise by villous growth between the pre-existing pattern of endometrial arteries. In these fetal villi, fetal arterioles run centrally to the tips before branching into a peripheral capillary network 75–125 μm in length (Tsutsumi, 1962), which carries blood back to veins at the base. The capillary blood therefore flows from the tips to the bases of the villi. Maternal blood, entering each microcotyledon (section 4.3.6a) over the rim of the cup-like structure, flows into a capillary network vascularizing maternal septa which have their tips between the bases of the chorionic villi. The flow is from the tip to the base of the septa; venous blood is collected in veins running round the base of the microcotyledon. In this species, therefore, there are clear morphological grounds for considering the gross blood flow to be countercurrent (Tsutsumi, 1962), but the microflow seems to be the cross and countercurrent system characteristic of apposed networks (Tsutsumi, 1962).

In the ruminants the sheep and goat have 10- to 100-fold longer villi forming much larger cotyledons than in the horse. Both maternal septa and fetal stem villi have central supply arteries which give off some branches as they run in opposite directions to their tips. Fetal and maternal blood passes back via capillary meshworks about 250 μm long in grossly countercurrent flow (Tsutsumi, 1962; Steven, 1966; Makowski, 1968).

The microflow arrangement has been controversial. It was initially suggested to be countercurrent (Barcroft and Barron, 1946) but reports of axial venules in the fetal terminal villi and maternal septa indicated that a crosscurrent system was equally likely (Figure 4.9; Tsutsumi, 1962; Steven, 1966; Makowski, 1968). A recent corrosion cast study of the goat placental vasculature has clearly demonstrated that the maternal and fetal capillaries are arranged in mixed cross and countercurrent microflow systems, network flow again (Figure 4.48; Leiser, 1987). A functional test has been applied by Metcalfe *et al.* (1965), who reversed the direction of flow on the fetal side of the placenta,

and found no major change in the rate of exchange of nitrous oxide from fetal to maternal blood; they concluded therefore that the flow must be cross-current. However, it is possible that flow reversal damaged the vascular beds, leading to altered transfer of the gas: a higher perfusion pressure was required to reach normal flow rates, when flow was reversed, and returning flow direction to normal did not restore normal vascular resistance. Measurements of transport using the non-metabolizable, freely diffusing substance, antipyrine, in chronically catheterized preparations have confirmed that the relationship is not exclusively countercurrent (see Battaglia and Meschia, 1986; Wilkening and Meschia, 1992).

The placenta of the cow is, in many respects, similar to that of the sheep (Tsutsumi, 1962) except that the placentomes are convex instead of concave, because of the greater complexity of the branching of terminal fetal villi. Convex placentomes are in fact sometimes found in sheep, particularly in late gestation; their formation can be induced by maintaining pregnancy in ovariectomized animals with administered progestagens (Alexander and Williams, 1966, 1968; Ricketts and Flint, 1980). It is not known, however, when the change from concave to convex placentomes occurs or what controls it. As in the ovine placenta fetal villi are vascularized by central arterioles running to their tips, branching to form many small lateral capillary beds, length 150–300 μm (Tsutsumi, 1962), which are drained by central venules. In cross-section these branches, which are longer and more prominent than those in sheep, resemble the arms of a starfish (Figures 4.9 and 4.49; Tsutsumi, 1962). The peripheral capillary bed in the maternal villi is supplied with blood from central arterioles and drained into venules at either the base of the villus or its centre. The relationship between maternal and fetal blood flows in the cow is therefore similar to that in the sheep and goat, with the exception that some maternal blood drains via central venules, whereas in the sheep the maternal capillary cascade (Silver and Steven, 1975) is said to be the only continuous channel back to the basal veins.

A detailed study of the endotheliochorial labyrinthine zonary placenta of the cat (Leiser and Kohler, 1983,1984) using scanning electron microscopy of corrosion casts shows that the fetal and maternal arterial systems deliver blood to opposite sides of the placenta before it drains back in what seems to be a grossly countercurrent arrangement. However, it is claimed that the capillary dispositions at the microflow level show that capillaries carry blood not in a direction opposite to maternal flow, but across it (Figures 4.9, 4.66 and 4.67). If this can be substantiated this labyrinthine placenta would have blood microflows which are principally cross-current (Leiser and Kohler,1984).

Vascularization of the haemodichorial rabbit placenta has been studied by dye dilution and angiography by Mossman (1926) and Carter *et al.* (1971), and has been reviewed by Carter (1975). The labyrinth (or zona intima) of the mature placenta is composed of a meshwork of trophoblastic tubules permeated by maternal blood. Fetal arteries penetrate directly to the maternal limit of this fetal tissue labyrinth before breaking up into capillaries which lead blood back towards the fetal side of the placenta to the fetal veins (Figure 4.72). Maternal spiral arteries pass through narrow sphincter-like regions in the myometrium before dilating to form large arterial sinuses located in the decidua; blood flows from these via four or five wide arteries to the fetal side of the labyrinth before draining back towards the decidua through the trophoblastic tubules of the placental labyrinth. From the tubules maternal blood enters large venous sinuses in the decidua which subsequently drain into the uterine veins. Countercurrent (gross) flow of maternal and fetal blood results from the fact that both arterial flows pass directly to the furthest

opposite sides of the labyrinth before draining back through the exchange area. As far as the maternal vasculature is concerned, this arrangement is consistent with the results of angiography (Carter *et al.* 1971). The maternal arterial sphincters are thought to represent the point just above which placental separation occurs at parturition, their purpose being to reduce maternal blood loss.

In the haemomonochorial villous placentas of man and Old World monkeys, as in the synepithelio- or endotheliochorial systems, the fetal arteries run to the tips of the fetal villi, here anchored in the maternal decidua, before draining back through a capillary network. However, unlike all the other placental arrangements, there are in man no channels to carry the maternal blood to the fetal surface, the maternal spiral arteries end abruptly at the maternal surface (Figure 4.88). A plausible explanation of how such a system works is as follows. The fetal villi are organized into groups called lobules or cotyledons, each of which has a hollow centre where villi are less densely packed. The lobules are situated over the openings of the maternal spiral arteries, which deliver blood into the hollow centre; in order to leave the intralobular spaces the blood flows radially over the villi into spaces between lobules (Wigglesworth, 1969). From these spaces venous blood leaves the placenta through venous openings in the decidual plate and interlobular septa (Figure 4.88). By angiography, puffs of maternal blood can be seen entering the intervillous space from the spiral arteries (Ramsey, 1967); pressure of blood may lead to a hollow-centred lobule by pushing villi outwards. Growth of the villi is thought to occur from the centre of the lobule towards the periphery, in the direction of blood flow; the most peripheral villi are reported to be more highly differentiated (Schuhmann, 1982). Solutes are removed from the maternal blood as it passes from the spiral arteries to the endometrial veins, thereby establishing a concentration gradient from the point of entry into the lobule to the exit. The diffusion barrier thickness of the human vasculosyncytial membrane does appear to be significantly reduced in regions where solute concentrations would be predicted to be lowest (Critchley and Burton, 1987; Burton and Tham, 1992). Arteriovenous shunting and blood stasis in the intervillous space are prevented by the velocity and momentum with which maternal blood enters this space and the villus architecture, which ensures mixing of highly oxygenated blood with that already present (Ramsey and Donner, 1980).

It has been suggested that the human placental villi represent a mop passively suspended in a bucket, but this does not explain the movement of maternal blood around the fetal villi in each lobule. It would be more realistic to imagine a brief separation to capillary dimension of the villi caused by each spurt of maternal blood, followed by closure of the intervillus 'capillary' spaces as the pressure drops – rather like water flow through fish gills. This system would prevent significant backflow towards the arterial orifices.

The structure of the fetal vessels in each villus is such that the terminal villi which protrude from the intermediate villi at random are vascularized by a network of peripherally located anastomosing capillaries arising from the central primary arteriole and draining into the central primary vein (Habashi *et al.* 1983). There is no evidence that the capillaries in the terminal villi have any consistent spatial relationship to the mean direction of blood flow in the intervillous space, so the maternal and fetal blood microflows presumably have a mixed or random directional relationship to one another (Figure 4.88).

Placental vascular architecture and placental function

Various functional parameters measured in different types of term placenta are summar-

ized in Table 4.1, in which placentas are arranged in order of the number of tissue layers separating maternal and fetal blood. Correlation of structure and function in the different placental morphological types should be simplest with diffusible gases and solutes where, given similar concentration gradients, the rate of transfer depends directly on the interhaemal diffusion distance and the area of the exchange surface.

The permeability of a placenta to blood gases can be determined directly by measuring the transfer of non-metabolizable carbon monoxide or estimated from morphological measurements (Mayhew *et al.*, 1984). The results show that the rate of fetal oxygen utilization is never limited by the oxygen diffusion capacity of the normal placenta. The range of values found in different species (7–45 ml/min/mmHg per kg of placenta) bears no simple relationship to the range of placental interhaemal structure. One problem here is that it is very difficult to determine the average interhaemal distance since the degree of indentation of fetal (or maternal if present) layers by the capillaries varies considerably both within the same placenta (Figures 4.25 and 4.44) and between placentas of different species. This indentation means that the minimum interhaemal distance in the epitheliochorial six-layered pig placenta may be very similar to that in the human haemomonochorial three-layered placenta, but the average interhaemal distance is much greater in pig than in man. Which value, minimum or average, is the more relevant is difficult to determine. It is possible that in the pig placenta there are enough closely adjacent lengths of maternal and fetal capillaries separated by the minimum distance to provide sufficient respiratory exchange for the fetus. The average interhaemal distance would not then be relevant. There are statistical treatments which are said to give more realistic estimates of the interhaemal barrier thickness (Critchley and Burton, 1987; Burton and Tham, 1992). There is some evidence for a local specialization of structure and function in the human placenta. Here the areas of minimum interhaemal separation, the vasculosyncytial membrane, are more extensive in regions where the oxygen concentration is plausibly suggested to be lower (Critchley and Burton, 1987). Whether such an arrangement is also found in other types of placenta remains to be demonstrated.

Differences in the transport capacity of various placental types clearly do not determine fetal growth rates, because as shown in Table 4.1 these are as high in the pig and the mare (epitheliochorial) as in primates or the guinea pig (haemomonochorial). Other factors that potentially influence placental efficiency are villous surface area, the size of the placenta relative to the fetus and placental blood flow rates. However, none of these parameters alone appears to correlate with fetal growth rate. Calculation of villous surface area at term per kg fetus from the data of Table 4.1, shows that this parameter varies widely, independently of fetal growth rate, from 300 cm^2/kg in the mare, to 10 400 cm^2/kg in the cat (Baur, 1977). Fetuses with compact placentas do not consistently grow faster than those with diffuse placentas, although compact placentas always have a higher villous surface area per gram of tissue.

Among the species in which fetal blood gases can be measured accurately, the mare has a markedly higher rate of transfer of oxygen into fetal blood, as judged by Po_2 (maternal vein minus fetal vein) than ruminants or the pig. This cannot be accounted for by a particularly high oxygen affinity of fetal haemoglobin compared with maternal, or to a high fetal haemoglobin concentration, or a high fetal arterial oxygen saturation, since these three parameters are similar in all species investigated. Unlike other species, neither the horse (Kitchen and Bunn, 1975) nor the pig has a special 'fetal' form of haemoglobin. It has been suggested (Silver and Steven, 1975; Steven and Samuel, 1975)

that the efficiency of gas exchange in the equine placenta reflects the countercurrent nature of the vascular architecture and the close proximity of the fetal and maternal capillaries.

The similar high gas-exchange efficiency of the guinea pig placenta may also be due to the suggested countercurrent flow (P. Kaufmann, 1981) plus the high fetal–maternal haemoglobin–oxygen affinity ratio. These are probably both adaptations to the lower oxygen availability at the high altitude at which these animals live in the wild in South America.

As the placenta grows the mother has to increase her blood volume and cardiac output in order to perfuse the new organ. The higher the blood flow through the maternal placenta in millilitres per minute per kg of fetus (see Table 4.1), the greater the demand on the maternal vasculature. Animals with haemochorial placentas have the advantage here since they consistently show lower maternal placental flow rates (100–160 ml/min/kg) than those with epithelio- or synepitheliochorial placentas (250–330 ml/min/kg). The last two types must also provide extra maternal energy to fuel active transport across the two maternal layers, endothelium and uterine epithelium, which are absent in haemochorial placentas. In all placental types on the fetal side the blood flows in endothelium-lined channels. Since the range of umbilical flow rates in haemochorial placentas from guinea pig (64 ml/min/kg fetus) to man (208) (Table 4.1) spans nearly all of the synepithelio- and epitheliochorial values from pig (115) to cow (232) there would seem to be no simple relationship between structure and function in this respect.

As well as total blood flow, the rate of fetomaternal exchange will depend, at least to some extent, on the speed of the flow through the placenta. It has been suggested that the slower the rate the more efficient the transfer, and structures have been identified in haemochorial placentas which are said to be designed to produce **local** maternal blood stasis (Enders, 1965). There is no doubt that in many haemochorial placentas the total maternal blood in flow is slowed by the development of arterial sinuses (rabbit) or grossly widened arterial vessels (guinea pig, man). There is also some evidence for intermittent rather than continuous blood flow in primate placentas. How relevant these flow rate changes are to efficiency of maternofetal transfer and whether there is an optimal flow rate remains to be determined.

Transport of hydrophilic, non-diffusible compounds requires carrier systems to allow them to cross each membrane barrier. Rates of transfer have been shown to be inversely proportional to the number of interhaemal membranes, which would suggest a similar frequency or equivalent capacity of carriers in each membrane. Sodium, for example, is transported 300 times faster in human (four membranes) than in pig (eight membranes) with the cat (six membranes) in an intermediate position (Moll, 1985).

However, what direct evidence there is indicates local specialization rather than uniform distribution. In the pig, Firth *et al.* (1986a,b) have shown that the Na^+,K^+-ATPase-based sodium transport system appears to be concentrated in the apical trophectodermal membranes of the fetal areolae. In human there are differences in carrier systems between microvillar and basal membranes of the syncytiotrophoblast (Shennan and Boyd, 1987; Vanderpuye and Smith, 1987) and the latter shows considerable local elaboration (Figure 4.87). Thus, within the basic constraint of the number of barrier membranes, there may be considerable localized differentiation of transport functions which are only now becoming apparent.

Control of placental blood flow

Most organs are capable of regulating their blood flow (the process known as autoregula-

tion), so as to maintain a constant rate (ml/min) of perfusion during periods of altered blood pressure. This process which involves local control over dilatation or constriction of arterial precapillary sphincters, would not be expected to occur in haemochorial placentas, which lack post-myometrial vessels with such sphincters. However, the premyometrial uterine arteries are responsive to systemic agents acting on vascular beds (Greiss and Anderson, 1970; Ford *et al.*, 1977) and, since maternal placental flow accounts for a high proportion of uterine blood flow, maternal placental flow can be modulated through effects on these vessels. Thus, Venuto *et al.*, (1976) showed that maternal placental blood flow was maintained at approximately 25 ml/min in haemochorial rabbits when mean arterial pressure was reduced from 135 to 65 mmHg, and C. Bell (1972, 1974) has described uterine vasodilatation following uterine nerve stimulation in the pregnant haemochorial guinea pig. However, in the synepitheliochorial sheep placenta, which has both precapillary sphincters and systemic agents affecting premyometrial vessels, there is no evidence for maintenance of a constant rate of perfusion; Greiss (1966) showed that, during the last 30 days of gestation in the synepitheliochorial sheep, uterine blood flow is linearly related to central arterial pressure over the range 10–110 mmHg (arterial minus venous pressures), and Anderson and Faber (1982) showed that this is also true for fetal placental flow over the range 25–45 mmHg, in chronically catheterized fetal lambs.

Although uterine arterial blood flow may not change with changing maternal blood pressure in all species, there is evidence that local factors may control the ratio of maternal to fetal blood flows in the placenta. Rankin and his colleagues (see Rankin and McLaughlin, 1979) have drawn an analogy between the placenta and the lung in this respect; in the lung compensatory mechanisms exist to maintain a constant ventilation–perfusion ratio following local vascular embolism or hypoxic alveolus. It is clear that mechanisms serving a similar function exist to control blood flow in the placenta; when part of the umbilical blood supply is occluded in sheep and rabbits, maternal blood flow to that region (as measured with microspheres) eventually falls (Rankin and Phernetton, 1976); furthermore in the normal sheep placenta maternal–fetal perfusion ratios are similar throughout the organ although the gross flow to various parts of the placenta varies (Rankin *et al.*, 1970; Rankin and Schneider, 1975). Rankin (1978) and Rankin and McLaughlin (1979) have suggested that PGE_2, which is produced in large quantities by the fetal placenta in the sheep (Challis *et al.*, 1976; M.D. Mitchell and Flint, 1978a), may be involved in regulating the ratio of maternal to fetal flow, by exerting opposite effects on different sides of the placenta. Such a mechanism would not work in a haemochorial placenta with no arterial precapillary sphincters capable of responding to vasoactive compounds; in synepitheliochorial placentas, such as that of the sheep, it would depend on the PGE_2 diffusing very considerable distances from the fetal vasculature. An alternative function for fetal placental PGE_2 may be in maintaining the patency of the fetal ductus arteriosus (Coceani *et al.*, 1978). Other mechanisms by which placental blood flow may be altered over long periods of time must include angiogenesis and local necrosis; the latter could explain the effects of partial umbilical occlusion and the former the relatively constant maternal–fetal blood flow ratios referred to above.

Because of the proximity of the maternal and fetal placental vessels it is possible that a transient rise in pressure on one side might lead to occlusion of the other. This has been investigated experimentally in the near-term sheep placenta, in which it has been shown that, while an effect can be demonstrated in the isolated perfused placenta by applying

large changes in pressure (Bissonnette and Farrell, 1973; Power and Longo, 1973; Power and Gilbert, 1977), this cannot be confirmed in sheep with chronically implanted catheters (Thornburg *et al.*, 1976). A second form of mechanical control of placental blood flow, which has been investigated principally in man and rhesus monkeys, is that exerted by myometrial contractility; individual contractions of the kind associated with the early stages of labour lead to compression of the veins running through the myometrium so that flow of blood into the intervillous space is intermittently interrupted, with potentially dramatic effects on fetal well-being. The most usually monitored parameter which reflects this form of stress during labour is fetal heart rate, the transient fetal bradycardia associated with increased uterine pressure known as a type II dip (or late deceleration) being an indication of fetal distress (see Ramsey, 1967; Carter, 1975).

4.2.6 TYPES OF YOLK SAC ABSORPTION AND PLACENTATION

Four basic types can be characterized by reference to the origin of the fetal cellular layers they present to the maternal tissues. The association with the maternal tissues is rarely as close as in the chorioallantoic placenta, absorption being from the fluid (usually uterine) at the fetal surface. The fetal layers are different in each of the four categories and serve as important if usually transient sites of physiological exchange between mother and fetus.

(a) Bilaminar yolk sac or bilaminar omphalopleure

This is present in all species initially as the layers surrounding the yolk or forming the blastocyst (Figure 4.1). The trophoblast forms the outermost layer and there are ultrastructural indications of absorption by endocytosis. Delayed implantation blastocysts can survive up to 6 months at this stage in the uterine lumen. The trophoblast is usually cellular but may also form a syncytium and initiate implantation as in lagomorphs. The pole opposite the embryonic disc may persist as a functional yolk sac zone to term in marsupials and some insectivores (Figure 4.3).

(b) Trilaminar yolk sac (trilaminar omphalopleure or choriovitelline placenta)

The central mesoderm is richly vascularized and the external trophoblast usually forms an intimate apposition with uterine tissues so a true placenta is formed. This is found in certain fish (Figures 4.14 and 4.15), reptiles (Figure 4.16) and marsupials (Figure 4.17) as the major placental structure. Eutherians frequently develop an area of this structure which may be important at early stages of development following implantation but never persists as a functional structure to term (Figure 4.2). The external trophoblast is usually cellular, but occasionally this forms a syncytium also, for example at implantation in carnivores. Monotremes and marsupials (Metatheria) typically have exclusively cellular bilaminar and trilaminar areas which persist to release from the egg or term (Figure 4.17). The bilaminar areas show intracellular structures indicating endocytotic uptake, while the trilaminar areas are structured to minimize the diffusion distance between fetal and maternal circulations, thereby facilitating respiratory exchange (section 4.3.5c).

(c) Incomplete/partially inverted yolk sac

Invagination of the yolk sac sphere to form a cup brings the vascularized endodermal top (or embryonic) half of the sac against the outer non-vascularized bilaminar half, obliterating the yolk sac lumen (Figure 4.3). The bilaminar segment may persist up to term in some insectivores, bats and rodents but usually thins and degenerates before this. A characteristic of the bilaminar segment is the

presence of a robust acellular membrane – Reichert's membrane – between trophoblast and endoderm (Figure 4.3). In many cases this is the only continuous layer of the three. Recent evidence indicates that in the rat it is composed of basement membrane material produced by the endodermal layer (Fatemi, 1987; Mazariegos *et al.*, 1987). The trophoblast may transform into a discontinuous giant cell layer and invade the endometrium as in the rat and mouse or persist as a columnar epithelium as in the squirrels (Figure 4.2). There is no firm evidence that the bilaminar layer acts as anything other than a coarse filter between the mother and fetus. Large protein molecules pass across freely. The top segment of the yolk sac does function as a barrier with selective transport capability as described below.

(d) Completely inverted yolk sac

In many rodents, insectivores and bats the bilaminar segment of the yolk sac is either never formed or disappears at an early stage. This exposes the vascularized columnar endodermal cell layer ('inverted yolk sac', see Figure 4.3) to the uterine lumen or endometrium. A number of studies have demonstrated selective absorption and transmission of proteins across these cells (Bainter, 1986), which are sealed at their apices by tight junctions. This is the main route for transfer of intact maternal immunoglobulins for fetal immunoprotection in rodents, rabbits and guinea pig. There is no evidence that their coexistent chorioallantoic placentas have any similar function. Conversely, in primates with no functional yolk sac, the chorioallantoic trophectoderm is the immunoglobulin transporting epithelium.

There is a considerable range of elaboration of the 'inverted' endodermal layer in the various orders from a simple and loose association with a flat uterine epithelium (mouse, rat) to a profusely branched villous array very closely apposed to corresponding uterine projections – a true placental structure only found so far in one rodent, *Perognathus* (Mossman, 1987). Whether such placentas play a necessary or purely subsidiary role during gestation has yet to be established.

4.2.7 TYPES OF CHORIOALLANTOIC ABSORPTION AND PLACENTATION

In the chorioallantoic placenta throughout gestation the outermost fetal layer is invariably the trophoblast (trophectoderm, chorion if in association with somatopleuric mesoderm) or a trophoblast derivative. It is a remarkably versatile epithelium showing great capacity for invasion, cell fusion, hormone production, specific nutrient absorption, selective transport, active metabolism and ability to resist maternal immunological attack.

(a) Cyto- and syncytiotrophoblast

All trophoblast is initially cellular; in endotheliochorial and haemochorial placentas this cytotrophoblast provides a continuous mantle of syncytiotrophoblast by division and fusion of one of its daughter cells into the syncytium. In haemochorial placentas the daughter cells may develop into individual giant cells which can migrate into the maternal tissues; in synepitheliochorial placentas they form fetomaternal syncytia throughout pregnancy.

Evidence from *in vivo* and *in vitro* growth studies shows that syncytiotrophoblast can form only by fusion of cytotrophoblast cells. No nuclear division has ever been clearly demonstrated in placental syncytium in any species. In the human placenta all the syncytial nuclei investigated were shown to be diploid, whereas the cytotrophoblast nuclei ranged from diploid to tetraploid as would be expected of a dividing cell population (section 4.3.6d).

The formation of syncytiotrophoblast ex-

clusively from cytotrophoblast means the latter needs to be available throughout pregnancy to provide for the enormous increase in area found in all placentas examined. In most haemomonochorial placentas the cytotrophoblast (which lies between the syncytiotrophoblast and its basement membrane) is discontinuous from an early stage but is always available to support expansion. Where the cytotrophoblast itself forms the (cellular) haemomonochorial placenta growth in area is by simple division [in *Zapus* the jumping mouse (Figure 4.97), *Tadarida*, a bat (Figure 4.11), and hyrax (Figure 4.96)].

Haemodichorial placentas (rabbit; section 4.3.6d) can theoretically accommodate expansion once formed as long as one layer has some cellular constituents which can divide and fuse into either layer to support growth (Figures 4.76, 4.81 and 4.98). However, there is no experimental evidence for the production of one layer from the other and the relationship between the two is very different from that between cyto- and syncytiotrophoblast in the haemomonochorial placenta.

In the haemotrichorial placenta, of which all so far investigated have two inner syncytial layers and one outer cellular layer in contact with maternal blood (Figures 4.76 and 4.100) (Enders, 1965; King and Hastings, 1977), there is a similar growth problem. No evidence for any contribution from the cellular layer to the adjacent syncytial (middle) layer has been reported. There is good evidence that the three layers are formed at the very start of labyrinth development (Carpenter, 1972) and that there is an enormous increase in area of all three layers as this chorioallantoic placental labyrinth grows. To support this growth it seems likely that in both haemodi- and trichorial placentas each syncytial layer grows independently from occasional persistent cellular insertions or interruptions. In the absence of evidence for any nuclear division in the syncytium this is the simplest hypothesis but requires more definite confirmation than the occasional observations currently available (Davies and Glasser, 1968; Metz, 1980).

Once formed, the haemochorial placentas show increasing attenuation of their constituent layers as pregnancy progresses, as do all other types of placenta. Near term all show areas of trophoblast, syncytial or cellular or both, which are so thin that organelles are excluded (Figures 4.81 and 4.87). These areas usually overlie deeply indenting fetal capillaries and, presumably, greatly facilitate rapid fetomaternal exchange. The trophoblast also has a considerable capacity for endocytotic uptake of non-diffusible molecules, and the normal ultrastructural marker for this is coated vesicle formation from caveolae at the relevant plasmalemma. In di- and trichorial placentas caveolae are only found on layer II, and not on the loosely apposed fenestrated layer I, which is bathed in maternal blood. Layer I forms no barrier to serum or tracer molecules but does provide a baffle plate slowing down blood flow and producing relatively static regions immediately adjacent to layer II, which may, according to Enders (1965), facilitate endocytosis. Other regions of trophoblast past which maternal blood flow would be sluggish (judging by blood space architecture) also have a high incidence of coated vesicles. In many haemomonochorial placentas a lattice of narrow subsurface intrasyncytial channels is found, and this is in continuity with the maternal blood space (Figures 4.10 and 4.99; Enders, 1982).

Coated vesicles form from these channels, which may represent an alternative method of providing a static blood serum reservoir for efficient absorption. The channels are derived from the basement membrane of the maternal endothelium, which is displaced by syncytiotrophoblast invasion starting soon after implantation. The intrasyncytial channels with their fibrogranular content persist and grow in extent as the syncytium increases in area throughout gestation. In the bat, *Myotis*, autoradiographic studies indicate

that the structured glycoprotein/carbohydrate material in the channels (Figure 4.99) is synthesized by the syncytium (Cukierski, 1987). In all cases save one, the intrasyncytial channels form a closed system accessible only from the maternal blood space. The exception is the bat *Tadarida*, in which the channel content is clearly continuous with the basement membrane of the fetal syncytiotrophoblast (Figure 4.11).

Whether this forms a continuous conduit from maternal blood to fetal connective tissue remains to be determined.

The fibrillogranular material in the channels is analogous in origin and appearance to the interstitial layer ('membrane') found at the syncytial surface of the endotheliochorial (carnivore) placenta (Figures 4.68, 4.70 and 4.71). Although normally superficial, this interstitial layer is frequently penetrated by syncytiotrophoblast processes. A function exclusively as a fetomaternal barrier therefore seems unlikely and further functions relevant to transport seem feasible.

The continuity of a syncytial layer is considered to provide a better barrier to potentially harmful or toxic maternal cells and molecules than a cellular layer linked by tight junctions. The rarity of cellular haemomonochorial placentas supports this conclusion, but their existence again emphasizes the lack of any unique advantage of a syncytium.

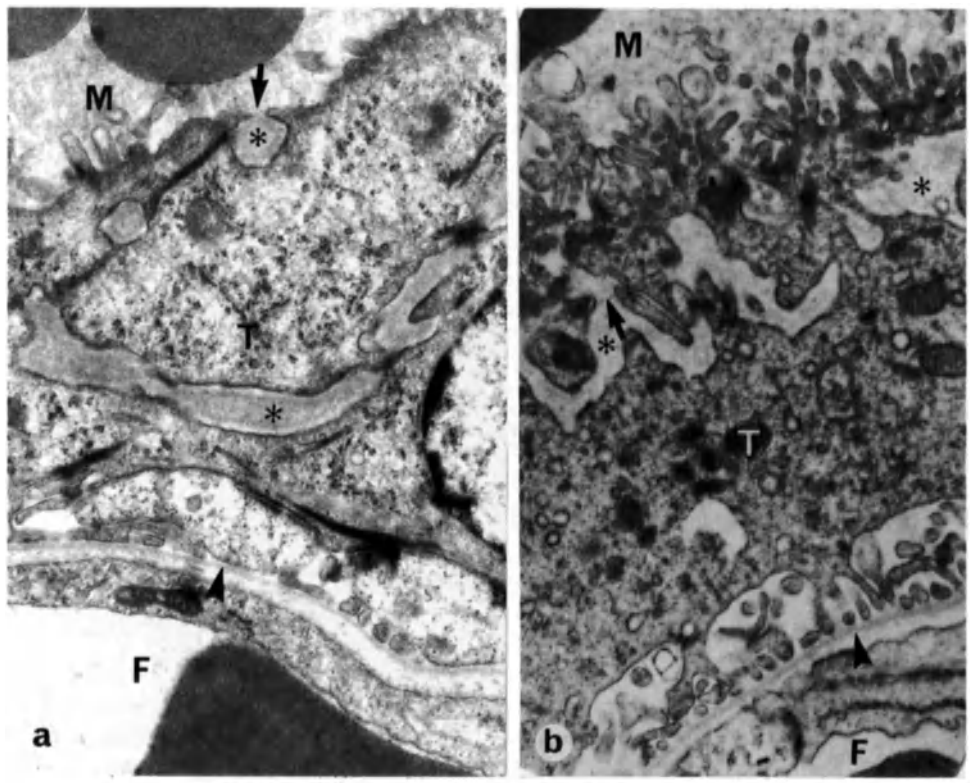


Figure 4.10 Haemomonochorial placentas with intrasyncytial lamellae (asterisks). In (a) the carnivore, hyena and in (b), the sciuromorph rodent, marmot, the lamellae (asterisks) are frequently open (arrows) to the maternal blood space (M) but no connection to the basement membrane (arrowheads) has been reported. (a) Hyena, mid-pregnancy; × 16 000. (b) Marmot, late pregnancy; × 15 200. (From Enders, 1965.)

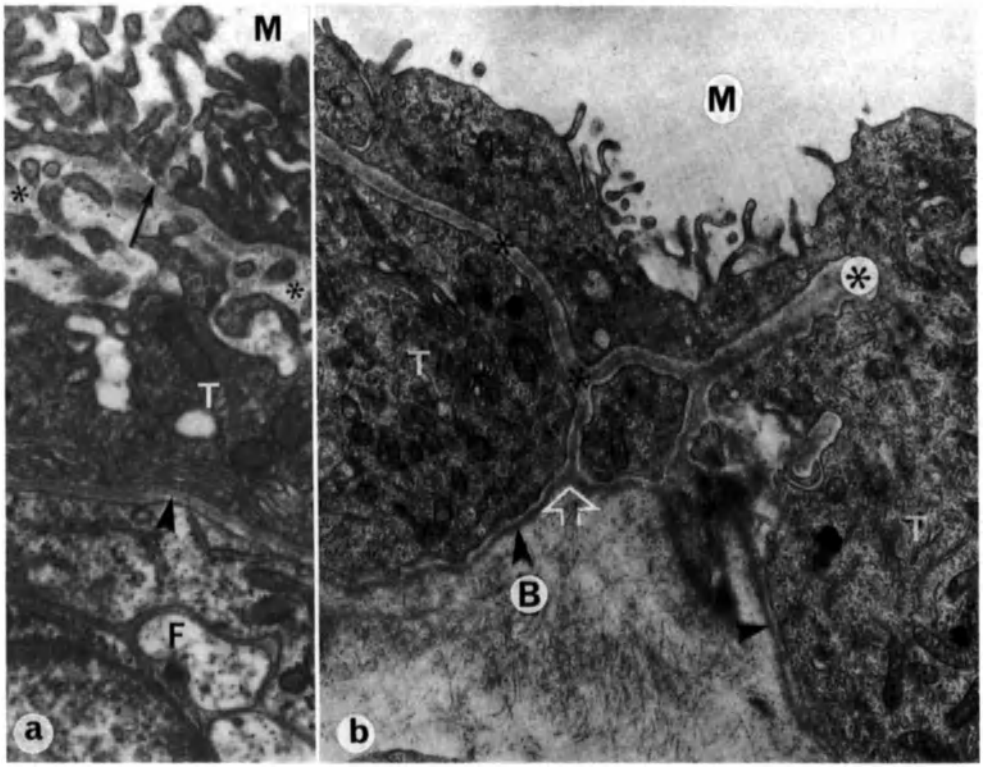


Figure 4.11 Haemomonochorial placentas with intrasyncytial lamellae (asterisks). In the sciuromorph chipmunk (a), the lamella is only open (arrow) to the maternal blood space (M) but not to the basement membrane (a, arrowhead) In (b) *Tadarida*, a molossid bat, the lamella and basement membrane (arrowhead, B) are continuous (open arrow) but here there is no evidence of any connection of the intrasyncytial lamella directly to the maternal blood space (M). F, fetal tissue. (a) Chipmunk, late pregnancy, × 21 500. (From Enders, 1982.) (b) *Tadarida*, late pregnancy, × 12 000. (From Stephens, 1969.)

In haemodi- and trichorial placentas the layers are linked by occasional tight junction remnants, frequent desmosomes and extensive gap junctions (Figure 4.81) (Metz *et al.*, 1976, 1978). The tight junctions may be remnants of those which linked the cells from which the syncytium formed and the desmosomes are needed to maintain the relationship between the layers. Gap junctions are normally considered to function by allowing the unimpeded passage of molecules below a certain size and thus to link cells biochemically for synchronous activity (uterine smooth muscle, for example). It has been suggested that in this placental context the gap junctions facilitate metabolite transfer across the layers. Tracer studies with lanthanum chloride and horseradish peroxidase clearly show that the healthy syncytial layers completely prevent transport in either direction of molecules of this larger size (Aoki *et al.*, 1978; Metz *et al.*, 1978) and any role of gap junctions in molecular transport from mother to fetus has yet to be demonstrated.

Although the syncytiotrophoblast shows no nuclear division it is very active in metabolism, synthesis and secretion. In man it has been shown to produce a wide variety of steroids and proteins including interferons (Bulmer *et al.*, 1990) and several hormones

(Figure 4.65), *in vivo* and *in vitro* (Patillo *et al.*, 1983). Syncytiotrophoblast in other groups contain an equivalent highly developed range of organelles and seems likely to play an equally important metabolic and endocrinological role.

Immunologically the syncytiotrophoblast surface facing the maternal blood in man and in mouse (the only two species investigated in detail so far) seems inert with no clear demonstration of any major histocompatibility complex (MHC) determinants. It has been suggested (Billington and Bell, 1983) that MHC antigen expression is obligately linked to cell division so that formation of a non-dividing layer would be one way of eliminating this expression with its attendant hazards.

Several advantages of a fetal syncytium in a placental context have been suggested: its plastic invasiveness, its synthetic versatility and capacity to secrete directly into the maternal compartment; its ability to form a continuous selective barrier which can include a considerable degree of regional differentiation or attenuation and; its immunological unresponsiveness and its inability to proliferate if fragments are released into maternal blood as human syncytiotrophoblast undoubtedly is (Covone *et al.*, 1984). However, there are examples of cellular trophoblasts which apparently perform just as well, so that syncytial change does not seem to be a prerequisite for successful placentation. Its widespread occurrence does argue that it may be better suited for carrying out the multifaceted requirements of a placenta than is a cellular system.

(b) Giant cells

Mononuclear giant cells are characteristic of rodent placentation and are formed from the surface cytotrophoblast of the conceptus during implantation and pregnancy. They are phagocytic and migratory and initially play an important role in enlarging the implantation chamber by eroding the superficial endometrial and decidual tissue including the capillary endothelium. Subsequent to implantation they form a loose sheath two to five cells thick around the conceptus through the interstices of which, on the antimesometrial side, maternal blood circulates. They are characteristically, though not exclusively, large (50–100 μm diameter) with large polyploid nuclei (16–32N, but 850N has been reported) (Barlow and Sherman, 1972; Ilgren, 1983). Visualization of individual chromosomes indicates that the giant cells usually only have the diploid number but each chromosome consists of multiple unseparated copies of its own DNA. This is polyteny rather than polyploidy and the distinction may have important functional consequences (Snow and Ansell, 1974; Bower, 1987; Varmuza *et al.*, 1988).

No evidence has been observed for cell division in giant cells with or without prior colchicine treatment. Incorporation of radioactive thymidine into giant cell nuclei is considered to be evidence of endomitosis, DNA replication without subsequent cell division.

Primary giant cells are defined as trophoblast cells which transform during and just after implantation on the antimesometrial and lateral (mural) aspects of the blastocyst external to Reichert's membrane. Secondary giant cells are formed a little later from the edge of the trophoblastic ectoplacental cone (M.H. Kaufmann, 1983). This is situated mesometrially and is a thickened cytotrophoblastic cellular plate which develops into the chorioallantoic placenta, producing from its growing edge secondary giant cells which are occasionally incorporated into the labyrinth.

There is great variation between rodent and lagomorph families in giant cell production, with murine rodents showing the largest and most characteristic forms. Their ultrastructure is unspecialized apart from the massive nucleus and quite different from the

adjacent spongiotrophoblast (Figure 4.82, rabbit). They frequently show evidence of phagocytosis, have little glycogen, contain both rough and smooth endoplasmic reticulum and a small Golgi apparatus. No cell junctions to other cells have been reported (Jollie, 1969; Kaufmann, 1983). The secondary giant cells form the boundary of the developing chorioallantoic placenta and play an important role together with the syncytiotrophoblast in the selective phagocytotic erosion of the maternal tissues essential for placental growth. They also form the only layer in one haemomonochorial placenta, that of *Zapus* (jumping mouse) (Figure 4.97). An endocrinological role is indicated by the finding that giant cells can produce steroids (Deane *et al.*, 1962) and placental lactogen *in vivo* and *in vitro* (Hall and Talamantes, 1984; Soares, 1987; Yamaguchi *et al.*, 1992). They share several characteristics with the endometrial cup cells in equids, and the ruminant binucleate cells, which have often been called giant cells in the past (Amoroso, 1952). All are transformed cells from the cytotrophoblast with specific secretions and capacity for migration (Hoffman and Wooding, 1993). There is one major difference in that ruminant and equid binucleate cells are diploid whereas rodent mononucleate giant cells are usually polyploid (Ilgren, 1983).

Rodent giant cells are immunologically specialized in expressing none of the MHC determinants on their plasma membranes (Lala *et al.*, 1983) and could therefore represent an immunologically neutral buffer zone when they form a continuous shell around the conceptus in early pregnancy. Most primary giant cells have degenerated by the time Reichert's membrane breaks down, and the majority of secondary giant cells also regress before the end of pregnancy. It has been reported that some giant cells which penetrate rather deeper than the others into the endometrium can survive parturition and persist until a subsequent pregnancy (Mossman, 1987). In humans all so-called 'giant cells' found in the maternofetal interface junction zone are syncytial, probably with diploid nuclei. They form part of the wide range of 'interstitial' trophoblast cells or syncytium scattered in the basal plate of the placenta (Hoffman and Wooding, 1993).

(c) Spongiotrophoblast

Rodent placentas usually have a definite zone between the giant cell layer and the base of the labyrinth referred to as the 'spongiotrophoblast', 'trophospongium' or 'junctional zone'. The tissue forms channels draining the maternal blood from the placenta but fetal capillaries do not penetrate this far (Figures 4.72, 4.77 and 4.82).

The spongiotrophoblast is derived from the outer layers of the original ectoplacental cone and is perfused by maternal blood from a very early stage of chorioallantoic placenta formation. It has been pointed out that it is in an excellent location to secrete fetal hormones or other effector molecules solely into the maternal circulation, and there is some evidence in the rat that it has a cytoplasmic structure significantly different from that in the contiguous labyrinth (Jollie and Craig, 1979). This does not seem to be true of the guinea pig (Figures 4.6 and 4.7) but in this case the uniquely structured subplacenta intervenes between spongiotrophoblast and maternal decidua (Figures 4.6 and 4.7). Its cellular form and extent vary considerably, ranging from a coarse mesh of syncytium in the guinea pig to a compact cellular layer in the mouse. Mossman (1987) suggests that it is homologous with the cytotrophoblastic shell at the base of the placental disc in primates. An immunological role has been postulated because in rat, mouse and man this trophoblast bears MHC antigens absent from the vast bulk of the haemochorial placental syncytiotrophoblast (Faulk and Hsi, 1983; Kanbour *et al.*, 1987), although there are dissenting voices (Chatterjee *et al.*, 1982).

(d) Endovascular trophoblast

In human (first trimester), hamster [10–11 days post conception (dpc)], rat and mouse placentas there is a considerable early invasion by the cytotrophoblast down maternal arteries supplying the placenta (Pijnenborg *et al.*, 1981). Migration proceeds almost to the myometrial level down those arteries which have already undergone decidual alteration, with an aggregation of pericytes around them and a swollen endothelium within. Migrating cytotrophoblasts then phagocytose and eliminate both the endothelium and the elastic lamina of the arteries, replacing them eventually with cytotrophoblast cells and their syncytial derivatives dispersed between tubular lamellae of fibrinoid. Slightly later (human, second trimester) there is a second cytotrophoblast migration even further, now into the (spiral) arteries of the myometrium. These successive migrations produce considerably widened tubes discontinuously lined with cyto- and syncytiotrophoblast which are permanently open, and unresponsive to vasoactive materials (Pijnenborg *et al.*, 1983; Hees *et al.*, 1987). These tubes can accommodate the vast increase in blood flow necessary to supply the exponentially growing fetus during the second half of pregnancy. Insufficient erosion and distension of the arteries has been shown to be correlated with failure of pregnancy in hamster and human (Faulk and McIntyre, 1983).

There is some evidence in man of an infiltration of lymphocytes and endometrial granulocytes around the distended arteries (Pijnenborg *et al.*, 1980). This may represent a maternal response to the invasion by the cytotrophoblast, which has been reported to bear MHC antigens (Faulk and Hsi, 1983).

(e) Fetomaternal hybrid syncytiotrophoblast

There is good evidence for fetomaternal cell fusion in three cases so far. Such hybrid tissue persists only briefly at rabbit implantation and in the chorioallantoic placenta of the marsupial *Perameles* (bandicoot) but is formed from implantation to term in all ruminants so far investigated.

In rabbit the blastocyst trophoblast forms a fetal syncytial 'peg' which fuses with a uterine epithelial cell or syncytium formed from the uterine epithelium (Figure 4.13) (Larsen, 1961; Enders and Schlafke, 1971a). By this means the blastocyst gains access to the endometrium to initiate formation of the chorioallantoic placenta. The maternal nuclei in this hybrid syncytium are said to disappear rapidly (although this aspect has not been closely studied) and the fetomaternal fusion is seen simply as a means of penetrating the uterine epithelial barrier.

Perameles is one of the few marsupials to develop a chorioallantoic placenta, and this forms only in the last 3 days of a 12 day gestation. At this stage the uterine epithelium consists of syncytia of limited extent (plaques), each containing several identical small nuclei. It is apposed to trophoblast cells each with a morphologically very different, large nucleus. Subsequently, the cellular trophoblast disappears and the syncytial plaques are found to contain both sizes and sorts of nuclei (Figures 4.17, 4.18 and 4.19) (Padykula and Taylor, 1976). No direct ultrastructural evidence for fusion of the apposed cellular and syncytial layers has been observed but this is the simplest explanation. The fusion allows the fetal and maternal blood capillaries to approach much more closely (see Figures 4.17, 4.18 and 4.19). The postulated fetomaternal tissue persists for the last 2 days of gestation when the fetus grows at a very rapid rate.

Fetomaternal fusion in ruminants is a much more persistent phenomenon (Wooding, 1982b). Binucleate cells develop in the predominantly uninucleate trophoblast just prior to implantation. They form 15–20% of this layer throughout gestation. When mature (i.e. with a full complement of their characteristic granules) they migrate out of the trophectoderm and fuse with the

apposed uterine epithelium to produce a persistent fetomaternal syncytium at the interface with the maternal endometrium (Figures 4.38, 4.42 and 4.43). This process continues throughout pregnancy (Figure 4.39), contributing to an integral layer of the placenta and/or delivering to the maternal tissue the granules which contain placental lactogen among other constituents (Figure 4.39). Further details and a discussion of the relevance of this system can be found in section 4.3.6(b) on synepitheliochorial placentation below and Wooding (1992). Other examples of fetomaternal fusion may well occur in other placentas, but without unequivocal markers it is difficult to recognize and verify such unexpected events.

4.2.8 DECIDUAL TISSUE, STRUCTURE AND FUNCTIONS

(a) Origins of decidua

Decidual tissue is usually restricted to those species with invasive, haemochorial placentation. It was initially defined as that part of the maternal endometrium which was shed at parturition. However, at partus little is left of this complex tissue which is now known to play a vital role from the start of successful conceptus development, and the definition has been widened considerably to take account of this (S.C. Bell, 1983; Kearns and Lala, 1983; Lala *et al.*, 1983; Glasser, 1990).

It is the non-glandular non-vascular cell populations of the endometrial connective tissue which form decidual cells, and this results in a considerable increase in the thickness of the gravid endometrium in early pregnancy. Decidual cell development is initiated either immediately below the uterine epithelium (rodents) or much deeper in the endometrium around the (spiral) arteries (lagomorphs, primates). The initial zones extend to produce a semicontinuous layer between the advancing trophectoderm and the myometrium.

By definition decidual cells are produced by steroid hormone-dependent division and differentiation of maternal uterine stromal cells (Glasser and McCormack, 1980; Tarachand, 1986). They form characteristic decidual cell areas found in significant extent only in orders with haemochorial placentation in which the trophoblast invades the maternal connective tissue: the insectivores, rodents, lagomorphs and anthropoids (Wynn, 1967). The areas are most prominent in early stages of pregnancy but vary considerably in extent in the different genera. In man there is a very extensive development, but in the closely related baboon the initial decidualization is morphologically much less obvious (Ramsey *et al.*, 1976), but all primates have extensive decidua by mid-pregnancy (Enders, 1991).

Decidual development is initiated by the arrival of a blastocyst or some artificial stimulus (oil drop, suture thread, electrical stimulation) in the hormonally primed (oestrogen plus progesterone) uterine lumen and follows a specific sequence (Figure 4.12).

In rodents the antimesometrial decidual cells develop first and form a very compact avascular zone which excludes most blood proteins from the uterine lumen containing the implanting conceptus (6–9 dpc) (M.B. Parr and Parr, 1986; M.B. Parr *et al.*, 1986; Christofferson and Nillson, 1988). Subsequently, the more loosely packed mesometrial decidual cells are closely involved in the fetomaternal interactions as the trophoblast invades to form the hemochorial placenta (8–12 dpc). Proliferation of the blood sinuses in the decidualizing mesometrial uterine stroma also facilitates placental growth (7–11 dpc). The areas of decidual cells rapidly increase from the uterine lumen outwards to the myometrium and can be conveniently visualized by the characteristic presence of alkaline phosphatase and periodic acid–Schiff (PAS) reactivity in the decidual cells (S.C. Bell, 1983).

Granulated decidual cells develop through-

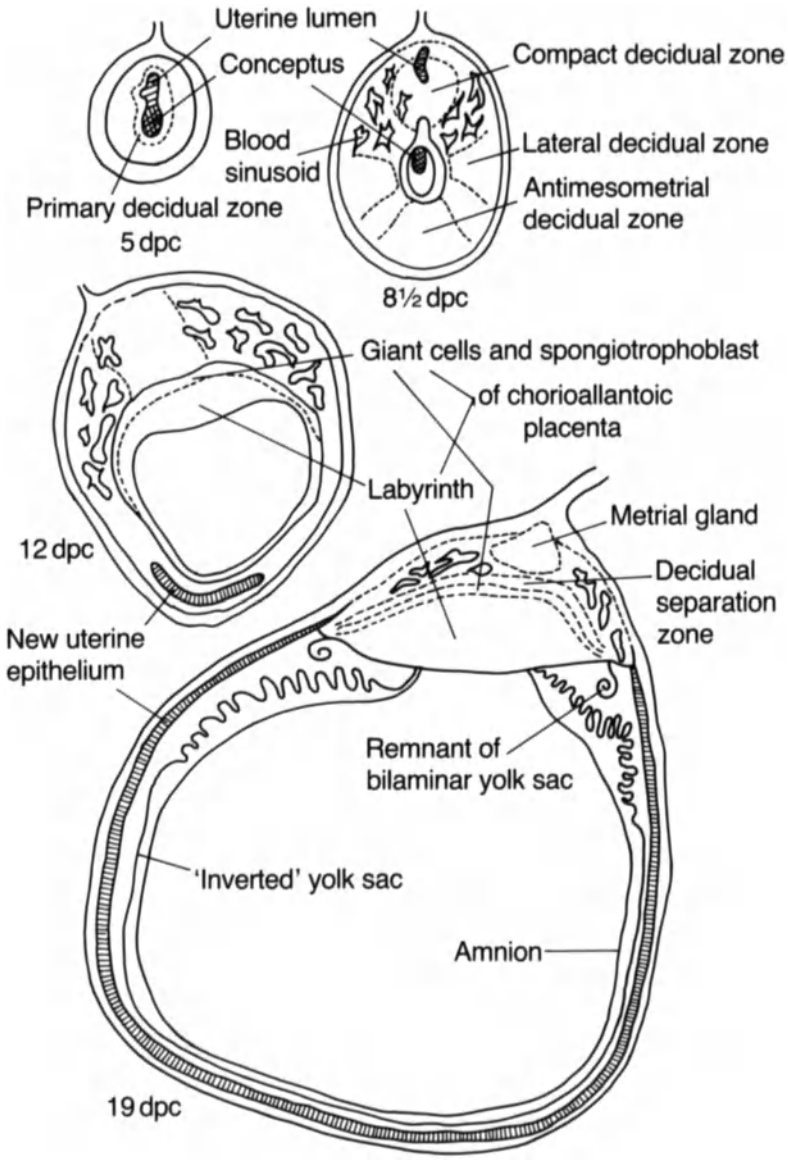


Figure 4.12 Scale drawings of the development of decidual and placental tissue in the mouse. (Based on S.C. Bell, 1983, and Rugh, 1975.)

out the mesometrial decidua but are initially concentrated around the blood vessels where they pass through the myometrium. This population eventually forms the metrial gland in rats and mice (section 4.2.8b). In man the conversion of stromal to decidual cells starts around the spiral arteries prior to implantation and spreads through the stroma to link up with the decidual area developing around the conceptus from 9 to 10 dpc (Figure 4.88).

Maximum development of the decidual cell populations occurs early in pregnancy, eventually occupying most of the uterine stroma and in mice and rats, completely surrounding the conceptus (Welsh and Enders, 1985). Cell division then largely ceases and the decidual cell layer is continually eroded by spontaneous necrosis and attenuated by growth of the placenta and fetus until at term it forms only a thin layer at the base of the placenta through which the placenta separates (Figure 4.12).

Deciduoma

The decidual tissue formed in response to an artificial stimulus is called a deciduoma, and is grossly similar to that induced by a blastocyst but has fewer regional specializations (Wadsworth *et al.*, 1980; O'Shea *et al.*, 1983). Deciduomas regress spontaneously in mice and rat (but not man) even if pregnancy is mimicked by maintaining the progesterone level artificially. This indicates that cells of the deciduoma have a predetermined lifespan and undergo programmed cell death, a process which is also seen to some extent later in pregnancy when sequential spontaneous death of the decidual cells facilitates the expansion of the conceptus and growth of the placenta. Decidualization therefore, does not produce a uniform population but a succession of coordinated differentiations of different cell types. There are also indications that specific lymphocytes and macrophages are recruited into the decidua from the circulation to perform functions related to pregnancy maintenance (Clark, 1985a). There is increasing evidence for a variety of functions for the decidua at different stages of haemochorial pregnancy: a physical and/or immunological barrier, a nutrient source, a hormone producer and a facilitator of placental growth and eventual release.

Decidualization and menstruation

The hormonal changes preceding ovulation produce correlated changes in the uterine stroma and epithelium. Usually such changes are restricted to tissue oedema and structural changes in the uterine epithelial cells (in ruminants and carnivores, for example). However, in humans and Old World monkeys the cyclic changes include considerable stromal to decidual cell development and proliferation of the spiral blood vessels. The subsequent cell necrosis and haemorrhage in this tissue as the progesterone level drops produces the menstrual discharge (Ramsey, 1982). The instructive similarities between menstruation, inflammation and decidualization have been pointed out in an excellent review by Finn (1986).

(b) Decidual cell structure

Stromal cells

The majority of decidual cells develop from the division in the endometrial stroma of irregularly shaped fibroblast-like cells with little cytoplasm and few organelles. Decidualization starts with the blood vessel pericytes in rabbits and humans and subepithelial stromal cells in rodents and characteristically involves a considerable increase in cell size (Amoroso, 1952; Mossman, 1987). The cytoplasm may develop meshworks of intermediate filaments, many free ribosomes, much glycogen and/or lipid droplets and numerous gap junctions with

adjacent decidual cells. Different areas (see Figure 4.12) also display their own characteristics. In rats and mice, antimesometrial decidua have very large closely packed cells, frequently binucleate or polyploid, with well-developed rough endoplasmic reticulum and Golgi apparatus and a low level of glycogen and/or lipid stores. Mesometrial decidual cells are smaller, more irregularly shaped and uninucleate, usually with rather more glycogen. Lateral decidual cells, among the rapidly expanding blood sinuses, have massive amounts of glycogen and are only occasionally binucleate (Welsh and Enders, 1985; M.B. Parr *et al.*, 1986).

In man there is a comprehensive decidualization throughout the uterine stroma but with less regional specialization than in rodents (Wynn, 1974; Schiebler and Kaufmann, 1981). There are three main types of human decidual cells. There are numerous 'small predecidual' swollen fibroblasts (50 μm × 5 μm) which have an equivalent low level of cytoplasmic organelle development and frequent 'large undifferentiated' decidual cells (100 μm × 25 μm). Scattered among these two predominant types are 'large differentiated' decidual cells (100 μm × 25 μm) with well-developed arrays of rough endoplasmic reticulum, many mitochondria, an extensive Golgi complex and large areas of glycogen and lipid. Human decidual cells are isolated from each other by an investment of fibrinoid and are linked by fewer gap junctions than are found in rodents (Boyd and Hamilton, 1970; Schiebler and Kaufman, 1981).

These cells form the basic structural matrix of the decidua. Other cells are invariably present (in pregnancy and deciduomata) which do not originate from stromal fibroblasts but are recruited instead from either the bone marrow (Gambel *et al.*, 1985; Krcek and Clark, 1985) (endometrial granulocytes equivalent to metrial gland cells), or the blood (decidual macrophages, Ia-antigen bearing cells, non-T suppressor lymphocytes) (Croy *et al.*, 1985). After the definitive placental structures have formed, the decidual area frequently also contains trophoblast derivatives: the occasional giant cell in rodents and large numbers of multinucleate trophoblast masses (known also as 'extravillous trophoblast') in humans as well as endovascular trophoblast in the blood vessel walls.

Non-fibroblast stromal cells

Endometrial granulocytes (metrial gland cells) Electron microscope and cell repopulation studies with irradiated mice and rats indicate that endometrial granulocytes develop from small round cells which originate from the bone marrow but divide and differentiate in the endometrial stroma (Peel *et al.*, 1983; Peel and Stewart, 1986). They can easily be distinguished on structural and staining criteria from blood eosinophils, basophils, neutrophils or mast cells. They are present in pregnant and pseudopregnant uteri (Peel *et al.*, 1979).

Mature granulocytes are the most frequent cell type after the fibroblast-derived decidual cells. They are often binucleate, and typically contain glycogen, rough endoplasmic reticulum and a large Golgi body which produces numerous membrane-bound granules. These are usually homogeneously electron dense, but some also have membranous inclusions (monkey, Cardell *et al.*, 1969; rat, Larkin and Flickinger, 1969; mouse, I. Stewart and Peel, 1977). In the light microscope the granules are eosinophilic and PAS positive and are said to contain lysosomal enzymes, IgG molecules synthesized in the granulocyte (B.S. Mitchell *et al.*, 1981), a protein, perforin, characteristic of killer and cytotoxic lymphocytes (E.L. Parr *et al.*, 1987), and the protein hormone relaxin, although this last localization has not been confirmed (Dallenbach-Hellweg, 1967, 1981). Human granulocytes show a weak natural killer activity which is enhanced by interleukin 2 (Ferry *et al.*, 1990)

and blocked by interferon-γ but not -α (A. King and Loke, 1993).

The granulocytes do not show phagocytosis, nor do they form intercellular gap, tight or desmosomal junctions with each other or with disparate cell types. In mice the cells average 50 μm in diameter and have a smaller number of larger (5 μm) granules compared with the rat (25 μm diameter, 2 μm granules) or man (12 μm diameter, 1 μm granules). The cells are found scattered in the mesometrial decidua from an early stage (mouse, 8 dpc) and in rat and mouse they collect around the myometrial blood vessels to form an accumulation in the mesometrial triangle between the myometrial layers which is referred to as the metrial gland (Figure 4.12) (For a comprehensive review see Peel, 1989.) Endometrial granulocytes are also found adhered to the walls of the placental labyrinth and decidual blood spaces. It has recently been suggested (I. Stewart, 1984) that such a granulocyte–trophoblast interaction may be relevant to their function, which may be related to non-specific immune suppression in the uterus and/or regulation of placental growth (Croy and Kassorf, 1989). Granulocytes have also been reported in the lungs of mice (I. Stewart, 1985).

The metrial gland reaches its peak size and placental vascular granulocytes their maximum numbers at 13–14 dpc in the rat. Between 15 and 21 dpc (term) there is a progressive loss of the cells by swelling and lysis and they release their cell contents including the granules into the connective tissue. This process has been referred to as holocrine secretion (Peel and Stewart, 1979) but seems more analogous to the 'programmed cell death' seen in other decidual cells, or cell regression in ovarian luteolysis. This would mean that the functional life of the cells is finished rather than that the release of the cell contents is the ultimate purpose of the process of granule development.

The cell debris is removed by macrophages, which are the probable precursors for the cells full of lipid droplets and phagolysosomes which progressively replace the granulocytes in the metrial gland region from 17 dpc. Some granulated cells may persist post term, but most regress long before parturition. There is no evidence for direct transformation of granulocytes to lipid-laden cells.

Decidual macrophages The evidence for this population comes from histological work and from tissue culture studies of mouse decidua (Hunt *et al.*, 1984). The cells have immunoglobulin (Fc) receptors, an ectoesterase enzyme on their plasma membranes and are phagocytic, suggesting an infiltrating macrophage type. This is corroborated by the absence of lymphocyte surface markers (IgG or Lyt antigens). The incidence of this cell type increases to a peak at 15 dpc in mouse, is strain dependent and low numbers seem to be correlated with poor fertility and a high conceptus resorption rate (S.C. Bell, 1983; Tawfik *et al.*, 1986).

Ia antigen-bearing cells Cells with a high level of Ia expression (MHC class II antigen) which lack lymphocyte or macrophage markers are typically involved in antigen presentation in the immune response sequence. Such cells have been identified in tissue cultures of mouse decidua from 14 dpc onwards (Elcock and Searle, 1985) and on sections of human decidua in early pregnancy (Oksenberg *et al.*, 1986).

Suppressor cells Characteristic cell populations have been isolated recently from mouse (mesometrial) (Brierley and Clark, 1987) and human (basalis) (Daya *et al.*, 1985) decidua which display suppressor activity against cytotoxic lymphocytes (CTLs) *in vitro*. Decidua lacking such cells cannot usually support a successful pregnancy. The cells are small and lymphocyte-like with eosinophilic granules and Fc receptors but lacking all lym-

phocyte surface markers (no Thyl, Lyt 1 or 2). They appear to produce a soluble factor capable of suppressing the interleukin 2 dependent expansion and subsequent attack by a CTL population directed against the fetal allograft. Fetal trophoblast is necessary for their recruitment (from about 7 dpc) into the decidua; they have not been found in deciduomata.

In the mouse, there is a population of larger cells showing similar suppressor activity found at implantation (4–6 dpc) which are also present in deciduomata. The detailed structures and localizations of these small and large 'suppressor' cells have yet to be clarified (Brierley and Clark, 1987).

'Decidual' structures in non-haemochorial placentation Extensive decidual cell development characterizes haemochorial placentation, but in some synepitheliochorial and endotheliochorial placentas specialized cells originating from uterine stromal cells are consistently incorporated throughout the definitive placental structure at the feto-maternal interface. The best example is the frequently binucleate giant cells in the cat placenta (Figure 4.68). Much smaller less obvious cells are also present in an equivalent position in the dog (Amoroso, 1952; Mossman, 1987). Cells of similar structure are found as giant pericytes around the maternal vasculature in the sheep and goat (Figure 4.69). These cells never form a coherent layer as in haemochorial placentas but their consistent presence suggests that they may play a role (producing a specific secretion?) in maintaining the delicate balance between fetal and maternal tissues. Decidual changes have also been reported in the pseudopregnant ferret, but the alterations are in the subepithelial uterine capillary endothelium rather than the stromal cells (Beck and Lowe, 1972).

Epithelial plaque Decidualization has been defined here as endometrial stromal cell differentiation. Epithelial plaque, which is always cellular, is formed by proliferation and development of uterine luminal and glandular epithelium dependent on the same hormonal regimen and enhanced in a similar way by contact with the blastocyst.

Plaque formation is characteristic of some monkeys whose implantation is superficial and whose early stromal decidualization is minimal (Rossman, 1940; Enders *et al.*, 1985; Owiti *et al.*, 1986). The epithelial proliferation is transient, starting at 6 dpc, and the plaque is engulfed and removed by the developing trophoblast by 20–22 dpc in the rhesus monkey for example (Figures 4.92 and 4.93) (Enders *et al.*, 1985). Prior to its obliteration it undergoes symplasmic degeneration. This life history is similar to that undergone by the surface epithelium in the uterus of carnivores and lagomorphs (Steer, 1971; Schlafke and Enders, 1975; Leiser, 1979). In these cases there is less initial proliferation but the symplasmic masses have to be absorbed in the same way by the trophoblast during the cellular remodelling involved in the trophoblast invasion of their maternal tissues. It seems probable that the symplasmic masses reported in the fetomaternal junctional zone of many placentas are derived from the epithelium of the glands rather than aggregated stromal decidual cells. Their production is the first stage in the reduction of the maternal tissue to merely a scaffolding of proliferating maternal blood vessels invested by a layer of vascular fetal trophoblast.

The extreme proliferation involved in plaque production can be seen as one of the ways of avoiding too rapid a penetration of the endometrium by the trophoblast (compare Figures 4.83 and 4.92). Plaque allows controlled access to the maternal circulation. Once this has been achieved superficial development of a purely fetal vascularized syncytium to form villi or a labyrinthine meshwork is all that is required to produce the haemochorial primate placenta. There is no need for deeper erosion of the endo-

metrium for in most Old World primates the maternal blood supply is restricted to orifices at the maternal face of the fetal tissue. Epithelial plaque formation is one way of achieving this controlled access to the maternal blood after superficial implantation: by contrast, interstitial implantation induces stromal decidual cell development (Ramsey, 1982). Both are systems for establishing a viable fetomaternal dialogue with a minimum of delay and stress to either party.

The variation in the balance between epithelial plaque and stromal decidual transformation (Ramsey *et al.*, 1976) is in sharp contrast to the similarity of the final placental structure. This may be taken as further evidence for the difficulty of rapidly establishing the fetomaternal dialogue: of balancing the nutritional needs of the embryo against the immunological sensitivities of the mother.

The same problems may be solved by what appear superficially to be very different morphological methods. In practice, small changes in mitotic rates of particular cell groups or phagocytotic capacity of the trophoblast might be sufficient to account for the differences.

(c) Functions of the decidual cells

Many functions have been proposed for decidua and it seems likely that there may be several roles it can play; which one is emphasized probably depends upon the specific demands of each particular animal group.

Isolation of the implanting blastocyst

In rodents with a small blastocyst there is a rapid initial decidual development of stromal cells immediately around the blastocyst (Figure 4.10). These cells become so closely packed, accentuated by both tight and gap junction formation, that maternal blood cells and proteins are prevented from reaching the blastocyst (M.B. Parr *et al.*, 1986). The effect is transient, lasting only from 6 to 9 dpc in the rat, and subsequently these early decidual cells die, to be displaced by the expanding conceptus. Reichert's membrane forms between the yolk sac ectoderm and endoderm coincidentally with the loss of this decidual barrier, and it has been suggested that this new acellular layer may take over the function of excluding harmful constituents of the maternal blood from the embryo (M.B. Parr and Parr, 1986).

Barriers to trophoblast invasion

Direct evidence that decidual cells act as barriers to trophoblast invasion comes from the observation that blastocysts transferred to subepithelial ectopic sites (kidney, testis, anterior chamber of the eye) penetrate into the non-decidualized connective tissue much more deeply than into uterine decidua (Kirby, 1963, 1965; Porter, 1967). Conversely, skin grafted onto decidualized uterine stroma is rejected much more slowly than skin in the non-decidualized uterus. The process of decidualization starts at the luminal surface, and the uterine epithelium, though rapidly lost, probably plays an essential role in mediating and amplifying the stimulus to decidualization (Kennedy, 1983). Uterine epithelium that is not receptive (i.e. that is not hormonally primed) is a much more complete barrier to blastocyst penetration.

A mechanical barrier results from the formation of a coherent decidualized cell layer by rapid division and expansion of the initially widely separated fibroblasts in the oedematous endometrial stroma (Glasser and McCormack, 1980; M.B. Parr *et al.*, 1986). The resulting decidual cells are often linked by gap junctions and are thus capable of concerted changes. It has been suggested that such a linked cellular barrier is so effective that expansion of the trophoblast is dependent on prior programmed cell death of the decidual tissue (Beaulaton and Lockshin, 1982). Certainly there seems to be a very close relationship between the two tissues in rodents (Welsh and Enders, 1985).

In man the decidualized cells are not so closely packed as in rodents and they frequently secrete a dense extracellular matrix consisting of collagen and glycosaminoglycans, which would also hinder trophoblast invasion (Boyd and Hamilton, 1970; Schiebler and Kaufmann, 1981). Such material is usually referred to as 'fibrinoid' and may form continuous sheets between fetal and maternal domains like Nitabuch's and Rohr's 'striae' at the base of the human placenta (Boyd and Hamilton, 1970).

However, such decidual cellular and extracellular barriers are rarely complete (Martinek, 1970) and elements of the trophoblast frequently penetrate well past the decidualized 'layer'. The interstitial and endovascular trophoblast of humans and rodents are examples of this breaching of the 'barrier'. The decidual layer may form an effective if partial barrier in some species (Bradbury *et al.*, 1965), but even then it is probably only part of an array of accommodations between mother and fetus to promote fetal survival.

A functional barrier may be provided by the secretion of specific proteins by decidual cells which either directly inhibit the protease activity of the trophoblast or compete locally for the growth factors such as insulin-like growth factor 1 (IGF-1) necessary for trophoblast growth. Bell and colleagues (S.C. Bell, 1979; S.C. Bell *et al.*, 1988) have published evidence for the production of decidual associated proteins and/or alkaline phosphatase which may serve such functions.

The loss of fibronectin from the surface of decidualizing cells may provide another functional barrier. As a result of the loss the trophoblast can no longer so readily form the close associations necessary for rapid and efficient killing or engulfment of the barrier cells (S.C. Bell, 1983).

Nutrient provision

One of the characteristics of decidualizing cells is the accumulation of glycogen and/or lipid stores, although this is not always found. It is generally assumed that this is produced for the eventual nutrition of the blastocyst. Amoroso (1952) noted that animals without decidual cells usually have many more uterine glands producing histotroph for blastocyst maintenance and growth.

Decidual cells phagocytosed by the trophectoderm would provide considerable readily convertible food stores very necessary to support the rapid growth of the conceptus. Presumably the barrier function of the decidua prevents the blood nutrients reaching the trophectoderm directly. Alternatively, the glycogen and lipid storage may be merely a measure of the senescence of the cells of the decidua which results in subsequent phagocytosis by the trophectoderm.

Hormone production

Prolactin There have been several reports of progesterone-dependent prolactin production by cultured decidual cells in man (Maslar *et al.*, 1986) and rodents, and histological studies indicate localization of prolactin to parietal decidual cells by immunofluorescence, (Braverman *et al.*, 1984; Al-Timimi and Fox, 1986; Bryant Greenwood *et al.*, 1987) and hybridization histochemistry (Xuan *et al.*, 1990; W.X. Wu *et al.*, 1991).

The amount of prolactin produced increases during pregnancy, and there is increasing evidence for a paracrine or autocrine role for this decidual prolactin in control of the osmotic balance at the fetomaternal interface (Meuris *et al.*, 1980; Gibori *et al.*, 1984). Control of decidual prolactin secretion is significantly different from pituitary prolactin because it is unaffected by bromocriptine (which blocks) or thyrotropin-releasing hormone (which stimulates) prolactin secretion from the pituitary (see Gibori *et al.*, 1984).

Prostaglandins and steroids Cultures of rodent and human decidual cells have been

shown to produce prostaglandins (Lala, 1989) and steroids, which have immunological potential as demonstrated by their effects on the mixed lymphocyte reaction (MLR) and cytotoxic lymphocyte (CTL) function. They are also implicated in the control of predecidual cell division, in the initiation and maintenance of decidual differentiation (Glasser and McCormack, 1980; Kennedy and Lukash, 1982; Clark, 1985b), and in the initiation and process of parturition.

Relaxin This hormone has been reported in human endometrial granulocytes and was suggested to be present in the rodent metrial gland (Dallenbach-Hellweg, 1967). Neither of these localizations has been confirmed. Sakbun *et al.* (1987) have reported an immunofluorescent localization to basal plate cells and the amniotic epithelium in human term placenta; and Bryant Greenwood *et al.* (1987) a localization to chorion laeve cytotrophoblasts and parietal decidua (see Sherwood, 1988, for an excellent review).

Immunological (functions) implications

1. Occlusion of lymphatics by adjacent decidual cell growth has been suggested to block the efferent immunological response to the foreign conceptus. However, there appear to be very few lymphatics in the uterine endometrial stroma of species which produce decidua so the importance of this effect is difficult to assess (Head and Seelig, 1984).
2. Soluble factors from cultured mouse decidual cells are said to have several local immunosuppressive functions (S.C. Bell, 1979). Such factors can recruit T and non-T suppressor cells, protect against natural killer (NK) and cytotoxic lymphocyte-mediated lysis and stimulate blocking antibodies rather than antibodies mediating rejection. The trophoblast is the primary source of these local immunosuppressive soluble factors, but the most recent informed opinion is that 'decidual factors can do almost all that trophoblast can' (Chaouat, 1987).
3. The decidual tissue forms a matrix in which immunologically active cells can modify the maternal response to the fetus (for a good review see Colbern and Main, 1991). There are reports of the presence of suppressor cells (mentioned in section 4.2.8c above) and macrophages and other Ia-positive cells which may process foreign antigens in such a way that the fragments induce tolerance rather than rejection (Clark *et al.*, 1984, 1986, 1987; Hunziker and Wegman, 1986). However, severe combined immunodeficient (scid) mice produce perfectly normal litters (Croy and Chapeau, 1990) although they lack B and T lymphocytes and have few NK cells, so such cells cannot be essential for normal pregnancy.

Release of the placenta

By definition the decidua is a hormone-dependent tissue layer at the fetomaternal interface autolysing on progesterone withdrawal to facilitate release of the placenta soon after birth of the young. Release of prostanoids by the autolysing cells may also play a part in the initiation and process of parturition (S.C. Bell, 1983). However, the wide variety of animals without decidua manage to separate their placentas successfully and in the European mole *Talpa* there is a well-developed decidua but the placenta remains *in situ* and is resorbed (Amoroso, 1952).

4.2.9 IMPLANTATION

Implantation is the mechanism by which the blastocyst establishes itself in the uterus, and the trophectoderm develops an intimate relationship with the uterine epithelium (M.B. Parr and Parr, 1989; Denker, 1993). This process is easily disrupted and most early preg-

nancy loss occurs during this time (Wilmut *et al.*, 1986). The process can also be suspended ('delay of implantation, embryonic diapause') at this stage, which serves to reduce the metabolic load on a lactating mother and to ensure that birth occurs at the most advantageous season (Enders and Given, 1977; Given and Enders, 1989).

The conceptus reaches the uterus in the morula stage surrounded by the acellular zona pellucida. It must then position itself correctly in the uterus, adhere to the uterine epithelium and possibly invade that epithelium in initiating the development of the characteristic placental structure. Recent studies have indicated that these structural changes are controlled by complex interactions between hormones, cytokines and their receptors (Ben Rafael and Orvieto, 1992; Mathialinagen *et al.*, 1992; C.L. Stewart *et al.*, 1992; Strickland and Richards, 1992; Wathes, 1992; De *et al.*, 1993).

(a) Position (spacing) in the uterus

In duplex uteri (rat, mouse, guinea pig, rabbit) the horns open separately into the cervix and blastocysts cannot pass from one horn to the other. Spacing in each horn is independent. In bicornuate uteri (pig, dog, cat, ruminants) the two horns are continuous and a conceptus ovulated on one side can pass to the other side prior to implantation (Figure 4.4).

In species with several conceptuses (polytocous), the blastocysts normally implant at evenly spaced sites along a uterine horn (Boving, 1971). How this spacing is achieved in the 2 or 3 days available is not clear. In the rabbit (duplex uterus) there appears to be no relationship to any pre-existing structure such as the scars of the previous pregnancy since conceptus number varies but the spacing is always even. If there is only one conceptus per horn it is always in the middle of the horn (Boving, 1971). In the pig and cat with bicornuate uteri, animals with a single ovary provide evenly spaced implantation sites in both horns and pig conceptuses of different phenotypes (colours) introduced in a set order randomize before implanting at an even spacing (Dziuk *et al.*, 1964).

These experimental observations indicate a considerable capacity for blastocyst migration along the uterine lumen and the most likely explanation is a passive movement propelled by the vigorous contractions of the uterine musculature. Inert beads of a similar size to a rabbit blastocyst are moved along if introduced into the uterine lumen at this time (4–6 dpc; Boving, 1971). However, the trauma of surgery makes this an unreliable experimental model. Presumably the conceptus would first need to be roughly spaced by vigorous muscular contractions instituted perhaps by substances present in the oviductal fluid entering with the conceptuses; and subsequently adjusted by local initiation of contractions and/or uterine growth by the individual conceptus.

This seems to fit with more of the observed facts than Mossman's ingenious 'leap-frog' concept. In this the first conceptus into the uterus attaches at the uterotubal end, modifying the surrounding uterine epithelium to inhibit further adhesion thus forcing the next conceptus to 'leap-frog' and pass further along to implant and so on.

However, all the above discussion refers to cat, pig or rabbit experiments, in which the blastocyst swells considerably (10–2000 × in the rabbit) during the positioning and adhesive phases. In the rat and guinea pig the blastocysts do not swell at this stage but are still evenly spaced in the uterus prior to adhesion. This may be achieved by muscular contraction since relaxin disrupts it (Pusey *et al.*, 1980), but there is no direct evidence for such movement of these tiny blastocysts (Boving, 1971).

In species with a single conceptus in the uterus (monotocous) or in each uterine horn (ditocous) there is also evidence for preferred sites of implantation. For example, multiple

ovulators such as the elephant shrew implant only a single conceptus in each horn, and some African antelopes ovulate on either side but implant predominantly in the right horn. No clearly identifiable receptive structures have yet been described (Mossman, 1987).

Most ruminants normally implant about halfway between the cervix and uterotubal junction but the caruncles at this site have no reported unique characteristics. It has been shown that cow and sheep blastocysts anchor themselves at their preferred site by trophectodermal outgrowths (papillae) into the mouths of the uterine glands (Figure 4.38) (Drieux and Thiery, 1951; Guillomot and Guay, 1982; Wooding *et al.*, 1982), but this does not explain how the site is initially selected.

There is now direct evidence for considerable conceptus movement in the uterus prior to implantation: the large horse blastocyst can be located *in vivo* with ultrasound, and it is reported that it moves freely between the two horns on 11–15 dpc but then implants caudally in one horn at 16–17 days (Ginther, 1979, 1983, 1984a,b).

There seems to be clear evidence for free conceptus movement, and also a consensus that positioning is complete before the zona pellucida is lost. Rupture of the zona then allows intimate adhesion between blastocyst and uterine epithelial surfaces.

(b) Adhesion

There are three main requirements for adhesion: loss of the zona pellucida, 'closure' of the uterine lumen, and development of trophectodermal pseudopodia (cellular or syncytial) which make membrane contact with the uterine epithelium.

The zona pellucida is ruptured and/or dissolved by a combination of blastocyst swelling plus the action of glycosidase and protease enzymes from uterine epithelium and blastocyst (Denker 1978; Denker *et al.*, 1978; Denker and Tyndale-Biscoe, 1986). The success of ectopic implantation demonstrates the non-specific nature of any uterine enzymes involved.

After rupture of the zona pellucida, closure of the uterus occurs by the resorption of fluid and development of muscular tone. These processes bring the uterine epithelium into an intimate relationship with the trophectoderm and this is accentuated in many species by swelling of the blastocyst.

Uterine closure brings the glycocalyces on the external surface of the trophectoderm and on uterine epithelial cells close together. Since both bear negative charges they will not readily adhere. Several cytochemical investigations have shown a generalized decrease in uterine epithelial surface change (measured by cationic probes) around the time of implantation (reviewed by M.B. Parr and Parr, 1989). There is also increasing evidence for change in the plasmalemma prior to the apposition between blastocyst and uterine epithelium. For example, erosion of the unusually thick glycocalyx can be demonstrated in the ferret (Enders and Schlafke, 1971b), changes in uterine epithelium luminal proteins have been shown in the rabbit (T.L. Anderson *et al.*, 1986; Hoffman *et al.*, 1990a) and alterations in the expression of carbohydrate epitopes demonstrated in the mouse (Kimber and Lindenberg, 1990). Specific lectins have been used to demonstrate pregnancy-dependent regional differences in binding to the blastocyst (Chavez and Enders, 1982) and to the uterine epithelium (Anderson and Hoffman, 1984; Anderson *et al.* 1986; Whyte and Allen, 1985; Anderson *et al.*, 1986).

However it is achieved, the fetal and maternal plasmalemmas characteristically finally adhere very closely at the implantation site. There is little clear evidence for fetomaternal tight or desmosomal junction formation despite several claims (Reinius, 1967; Potts, 1968; Schlafke and Enders, 1975; Tachi and Tachi, 1979). Evidence from freeze-fracture is required to adequately characterize the

nature of the adhesion. Examination of the blastocyst attachment to uterine epithelium *in vitro* may also provide useful information (Lindenberg *et al.*, 1989).

(c) Invasion and/or placental development

From the common start of flat apposition between maternal and fetal apical plasma membranes there are several different ways of initiating placental development. Schlafke and Enders (1975) categorized four basic types: simple interdigitation, displacement, fusion and intrusive implantation (Figure 4.13).

Interdigitation

In the pig (Dantzer, 1985) the flat apposition develops into an interdigitation of microvilli from trophectoderm and uterine epithelial surfaces. There is no loss of cells or any fetal or maternal syncytial transformation. Development of the placenta is essentially a vast increase in the area of fetomaternal apposed epithelia with their interdigitated microvilli.

Displacement

In the mouse, rat and hamster the apposition of the fetal and maternal cellular epithelia results in loss of integrity and subsequent delamination or sloughing of individual cells and sheets of the uterine epithelium which are then phagocytosed by the trophectoderm (M.H. Kaufman, 1983; Schlafke *et al.*, 1985; Bevilacqua and Abrahamsohn, 1989; Blankenship *et al.*, 1990).

This change in coherence of the uterine epithelial cell sheet may be induced either by contact with the trophectoderm or by isolation from the vascular supply by the coincident decidual cell development immediately below the uterine epithelial basement membrane. Whatever the cause it does not seem to be analogous to normal cell killing, since inhibition of RNA synthesis with actinomycin D completely blocks the uterine epithelial change (Finn and Bredl, 1973) and the trophectoderm then is reported to penetrate the uterine epithelium intrusively (see below). The results certainly suggest fetomaternal interaction rather than cytolysis and phagocytosis by an aggressive trophectoderm (M.H. Kaufmann, 1983).

This sort of concerted delamination of a large area of uterine epithelium has also been reported for the vespertilionid bats *Myotis* and *Glossophaga* (Enders and Wimsatt, 1968; Rasweiler, 1979; Kimura and Uchida, 1983, 1984).

Fusion

Evidence for fusion between trophectodermal syncytial protrusions and uterine epithelial cells in rabbits has been presented by Larsen (1961) and Enders and Schlafke (1971a). Fusion of cytotrophoblast cells produces a trophectodermal syncytium which forms numerous processes. These penetrate the zona pellucida and indent the apex of the uterine epithelial cells forming initially a flat apposition. Subsequently, the apposed membranes fuse to form a fetomaternal hybrid tissue. Evidence of fusion comes from the observation of chains of vesicles in regions where one would expect flat apposition. Also, cells linked to the adjacent uterine epithelium by the usual apical tight junction complex demonstrate cytoplasmic continuity with the overlying trophectodermal syncytium (Figure 4.13). Since at the same time the uterine epithelium itself is producing plaques of symplasm by loss of lateral membranes, it is difficult to follow the detailed outcome of the initial fetomaternal fusion. Fusion seems to be necessary to initiate trophectodermal invasion, but it is considered likely that the maternal nuclei and cytoplasm make only a transient contribution to the invasive trophectodermal syncytium (Enders and Schlafke, 1971a).

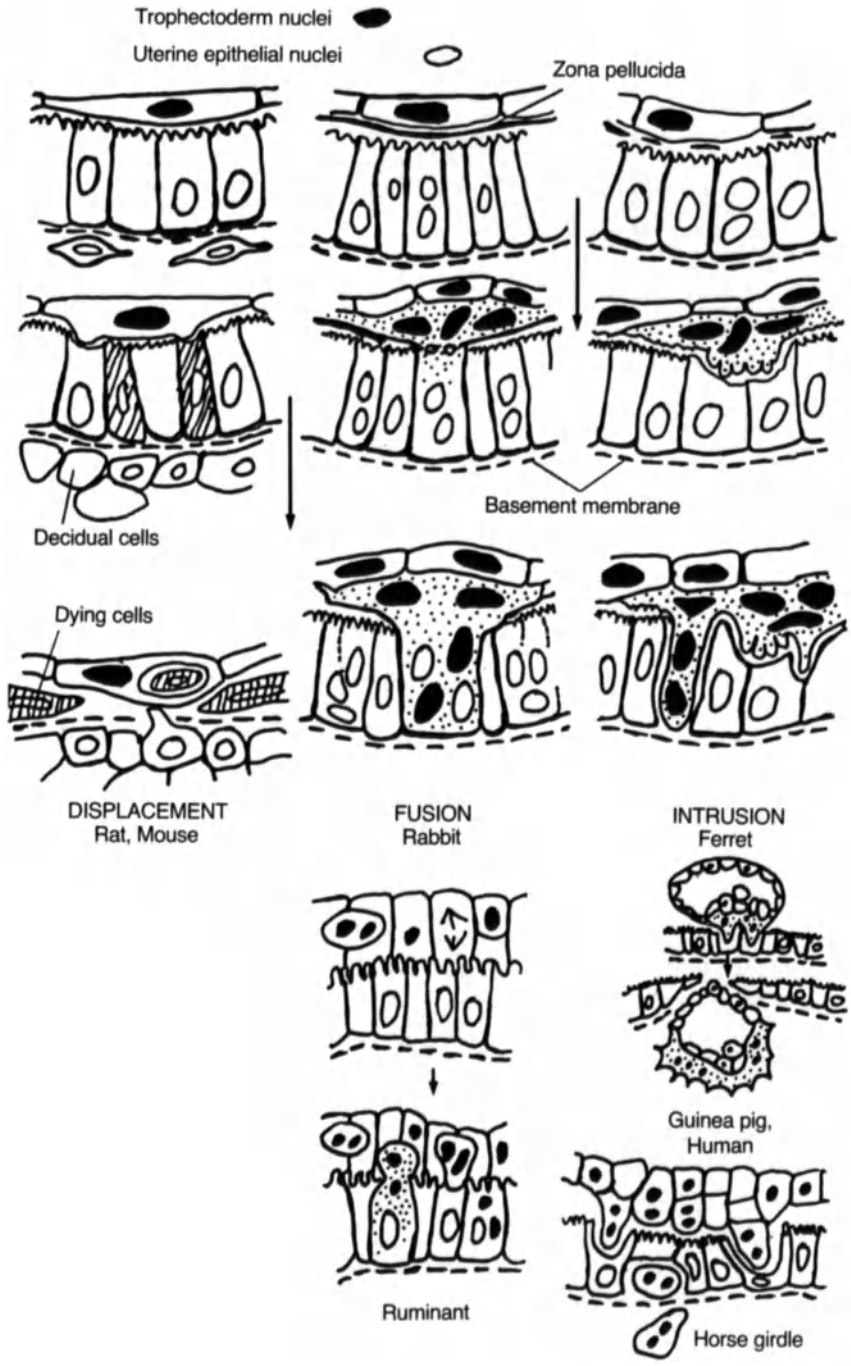


Figure 4.13 Classification of the different fetomaternal cellular interactions at implantation. (Based on Schlafke and Enders, 1975.)

In the ruminant trophectoderm, characteristic granulated binucleate cells (BNCs) are first produced just before implantation (Wimsatt, 1951; Wooding, 1982b, 1983). BNCs migrate to form a flat apposition with a uterine epithelial cell, and then fuse apically, finally providing a trinucleate cell within the uterine epithelium (Figures 4.13 and 4.38) (Wooding, 1982b). Evidence for the fusion comes from images of the two cells cytoplasmically continuous across the fetomaternal microvillar junction but linked by tight junctions to their epithelia (Figure 4.42) and the presence of BNC-derived granules containing placental lactogen hormone only in trinucleate cells in the uterine epithelium (Morgan *et al.*, 1987) (see section 4.3.6b for further details).

The final example of fusion comes from the marsupial *Perameles* (Padykula and Taylor, 1976). On about the ninth day of the 12-day gestation a limited area of trophectoderm cells, each with a characteristically large nucleus, comes into close apposition to the uterine epithelium which consists at this stage of plaques of syncytium containing numerous very small nuclei. Specimens a day or so later display no separate trophectodermal layer but the fetal and maternal blood capillaries are separated only by plaques of syncytium which contain a mixture of very small and characteristic large nuclei (Figures 4.17, 4.18 and 4.19). The simplest explanation for the apparent disappearance of the trophectoderm and two sizes of nuclei in the final syncytium is fetomaternal fusion but the details have yet to be investigated (see section 4.3.5c).

The advantages of fetomaternal hybrid tissue formation are obscure. It does offer rapid access through the uterine epithelium while maintaining its structural integrity; presumably some immunological camouflage is provided by the maternal component of the hybrid tissue. However, the problem of dual cytoplasmic control must be formidable, and the fact that the hybrids are produced by such dissimilar methods – fetal syncytium or cell fusing with maternal cell or syncytium – once again indicates that these cases are probably individual answers to specific problems rather than a general solution of the puzzle of fetomaternal accommodation.

Intrusion

This type is found in several families and is characterized by firm adhesion followed by penetration of trophectodermal processes through a healthy uterine epithelium without any signs of destruction of, or alteration in, the contiguous uterine cells (Figure 4.13). In the ferret syncytial processes pass between the cells, apparently forming tight junctions with them (Enders and Schlafke, 1971b). In contrast, in the horse individual binucleated trophectodermal cells are said to indent the cell apices away from the tight junctions and initially pass through the body of the uterine epithelial cell on their way to form the endometrial cups (Allen *et al.*, 1973). In both cases the uterine epithelial cells are subsequently surrounded and phagocytosed to allow a broader trophectodermal access to the endometrium.

The tiny blastocysts of the human and guinea pig form a trophectodermal syncytium at the site of apposition to the uterine epithelium (Enders and Schlafke, 1969; Boyd and Hamilton, 1970). This seems to facilitate rapid subsequent penetration of the entire conceptus into the endometrium, but the details of the process are unknown. The uterine epithelium subsequently closes over the implantation site.

Intrusion seems to require protrusions from syncytium or binucleate cells, but the means by which these negotiate the tight junction barrier remains to be elucidated. The ruminant BNC is the only cell so far demonstrated capable of passing through a tight junction while maintaining the junction intact, but the BNC is derived from that same epithelium (Morgan and Wooding, 1983).

Intrusion implantation suggests that the fetal trophectoderm can form tight junctions with the maternal tissue but there is no direct evidence for this in any system as yet.

As more EM studies are made it becomes clear that the categories above may not be exclusive. In the mouse, for example, there is an excellent electron micrograph which demonstrates that the first visible sign of implantation may be intrusion into or fusion with a healthy uterine epithelial cell (Figure 4.73) (M.H. Kaufman, 1983), and at the light microscope level similar penetration of the intact uterine epithelium has been reported after actinomycin D injection (Finn and Bredl, 1973).

In some marsupials there is a local penetration of an otherwise unchanged uterine epithelium by cellular trophoblast which could be by fusion or intrusion (Tyndale Biscoe and Renfree, 1987); a similar process has been reported in *Galago* (Butler, 1967). There are now three examples of fusion implantation; only one was known in 1975. This process may be more widespread than has been realized.

The problem is the transience of the initial cellular events and the lack of markers to distinguish fetomaternal fusion from intrusion. The ruminant BNC fusion with a uterine epithelial cell was recognized by several criteria. There are obvious BNC 'marker' granules; it is a cellular system so that the number of nuclei per hybrid cell can be counted; finally the tight junction between fetal and fetomaternal trinucleate cells (TNCs) can be logically explained by the migration and fusion hypothesis (Wooding 1982b, 1984).

Investigations of other systems are complicated by lack of markers and by considerable coincident syncytium formation so it is not practical to count nuclei. However, it should be remembered that tight junctions between fetal and maternal tissue [as reported, for example, by Leiser (1979) at cat implantation] may indicate fusion just as well as intrusion.

4.3 DEVELOPMENT OF EMBRYONIC MEMBRANES IN THE VARIOUS VERTEBRATE TAXA

4.3.1 FISHES

(a) Introduction

The fishes are non-amniotes which have evolved a wide variety of modifications for successful viviparity (Amoroso, 1960; Hogarth, 1976; Wourms, 1981; Mossman, 1987). The most widely used system is an increased secretion of nutrients into the ovarian or uterine cavity and absorption via the fetal gut or skin surface, but a number of genera have produced specialized structures, both embryonic and extraembryonic, some of which closely parallel amniote placentas.

(b) Classification

Fishes:

Class	Chondrichthyes (cartilaginous fish)
Subclass	Elasmobranchii, selachians

About half the 100 families have viviparous members; only two of these families (Carcharinidae and Syphonidae, including sharks, skates and rays) have either a yolk sac placenta (Figure 4.4) or trophonemata. The latter are long glandular extensions of the uterine epithelium producing abundant secretion (Wourms, 1981).

Class	Osteichthyes (bony fish)
Subclass	Actinopterygii

This includes more than 99% of all living bony fish. Fourteen out of the 425 families are viviparous, and five of these have structures ranging from fin elaborations, gut extensions (trophotaeniae) and expansions to extraembryonic pericardial sac elaborations analogous to the vertebrate amnion and chorion (Figure 4.15).

It is clear that fish viviparity has evolved many times in many different genera (Amor-

Table 4.2 Dryweight increases during gestation in fishes

Species	*Percentage change in dry weight of conceptus*	*Reproductive pattern*	*Uterine secretion*
CHONDRICHTHYES; Cartilaginous fish			
I OVIPAROUS			
Scyliorhinus caniculus	−20	oviparous	none
II VIVIPAROUS			
Squalus acanthias	−40	yolky egg	serous
Mustelus mustelus	370	yolky egg	mucous
Dasyatis violacea	1,600	trophonemata	lipid
Mustelus canis	1,050	placenta	mucous
Gymmura micrura	4,900	trophonemata	lipid
Odontaspis taurus	1,200,000	intrauterine cannibalism	
OSTEICHTHYES; Bony fish			
I OVIPAROUS			
Salmo vivideus	−40	oviparous	
II VIVIPAROUS			
Poeciliopsis monacha	−40	yolky egg	
Poecilia (lebistes) sp.	0	yolk, small pericardial sac	
Heterandria formosa	4,000	large smooth pericardial sac, simple	
Anableps dowei	800,000	follicular placenta large specialised pericardial sac	
Ameca splendens (Goodeidae)	15,000	villous follicular placenta	
Embiotoca lateralis	20,400	trophotaeniae	
Jennynsia sp.	24,000	fin extensions, gut expansion trophonemata	

oso, 1960, Wourms, 1981). A decrease in the amount of yolk after retention in the body of the mother necessitated an efficient system of nutrient transfer. In fishes, the placenta is only one, and not necessarily the most efficient, solution to the problem. In fish physiology a rough measure of the efficiency of viviparity is taken as the percentage increase or decrease in organic material between egg and neonate (Table 4.2).

(c) Chondrichthyes

In Table 4.2 it can be seen that retention in the uterus does not necessarily offer any nutrient transfer advantage (*Scyliorhinus* versus *Squalus*) unless the organic content of the uterine secretion is increased (*Squalus* versus *Mustelus mustelus*). Development of trophonemata, uterine glandular extensions which may be in close relationship with the fetal mouth or gills, further augments the percentage increase (*M. mustelus* versus *Dasyatis*) and a true trilaminar yolk sac closely apposed to an expanded semivillous uterine epithelium is almost as effective (Cate-Hoedemaker 1933; Schlernitzauer and Gilbert, 1966) (*M. canis* versus *Dasyatis*). However, a richer uterine secretion plus trophonemata can be three or four times as good as a yolk sac (*Gymmura*

versus *M. canis*), and a species in which the surviving uterine tenant has eaten all its originally numerous siblings (*Odontaspis*) has no rival measured on this criterion.

Yolk sac placenta

In several of the skates and rays after the yolk is exhausted the trilaminar vascularized yolk sac is then modified to serve a placental function. Such species have very lengthy gestation times. In *Mustelus canis* (*laevis*) which has been most closely investigated structurally (Cate-Hoedemaker, 1933), the placenta forms after a 3 month dependence on yolk and is functional for another 9–10 months (Figure 4.14).

The uterus develops shallow folds around each embryo and within the fold the yolk sac trophoblast forms with the uterine epithelium a localized intimate association which subsequently increases in area by folding. A shell membrane, or derivative thereof, persists between the maternal and fetal epithelia until term, but there is a considerable decrease during pregnancy in the thickness of the epithelia and the shell membrane. In addition, the blood capillaries eventually deeply indent the epithelia, reducing still further the separation of fetal and maternal blood (Figure 4.14). Cate-Hoedemaker (1933) suggested that there was a syncytial change in both epithelia, but this has yet to be verified electron microscopically. A more recent tracer and ultrastructural study of the yolk sac placenta of *Carcharhinus* (Hamlett and Wourms, 1984; Hamlett *et al.*, 1985) demonstrated the ability of the trophoblast to absorb proteins endocytotically and of the uterine epithelium to mediate transfer, but capillary indentation of the epithelia was not so marked as in *M. canis*. There is a complete range from the firmly adherent structurally modified yolk sac placenta of *Mustelus canis* to the transient non-specialized association of yolk sac trophectoderm and uterine epithelium (across a fairly thick shell membrane) reported in *Squalus acanthis* (Jollie and Jollie, 1967). In some cases (*Scoliodon*) very complex elaborations of the yolk sac stalk surface (appendiculae) are present as well as a placenta (Figure 4.14). These fetal appendiculae, like the glandular extensions of the maternal uterine epithelium (trophonemata), may also play an important role in fetomaternal nutritive exchange in other families but, since the tissue association is very loose, as far as it has been investigated, a placental relationship cannot be assumed (for a recent review see Hamlett, 1989; Hamlett *et al.*, 1993).

Thus, in the Chondricthyes a true yolk placenta with considerable specialization for haemotrophic exchange has been developed, but the vast majority of viviparous species rely on histotrophic nutrition with only a few structural adaptations to facilitate this.

(d) Osteichthyes

The bony fish do not have true uteri and viviparous development takes place either in the ovarian follicle or, after follicular rupture, in the ovarian cavity (Turner, 1947; Amoroso, 1960; Wourms, 1981). Table 4.2 demonstrates that again the level of maternal organic provision is greatest when facilitated by specialized fetal and/or maternal structures. Unlike the Chondricthyes the yolk sac is not developed further than its function as a yolk store; but in parallel with the Chondricthyes some species do have gland-rich trophonemata which reach from the uterine surface into the mouth or gill cleft of the embryo. The other structures, pericardial sac, trophotaeniae or fin extensions, are unique to a few families of the Osteichthyes (Figure 4.15) (Schindler and Hamlett, 1993).

Pericardial sac

In three families (Anablepidae, Poeciliidae and Goodeidae) gestation starts in the follicle. In early development the exocoelomic cavity around the heart extends to form an

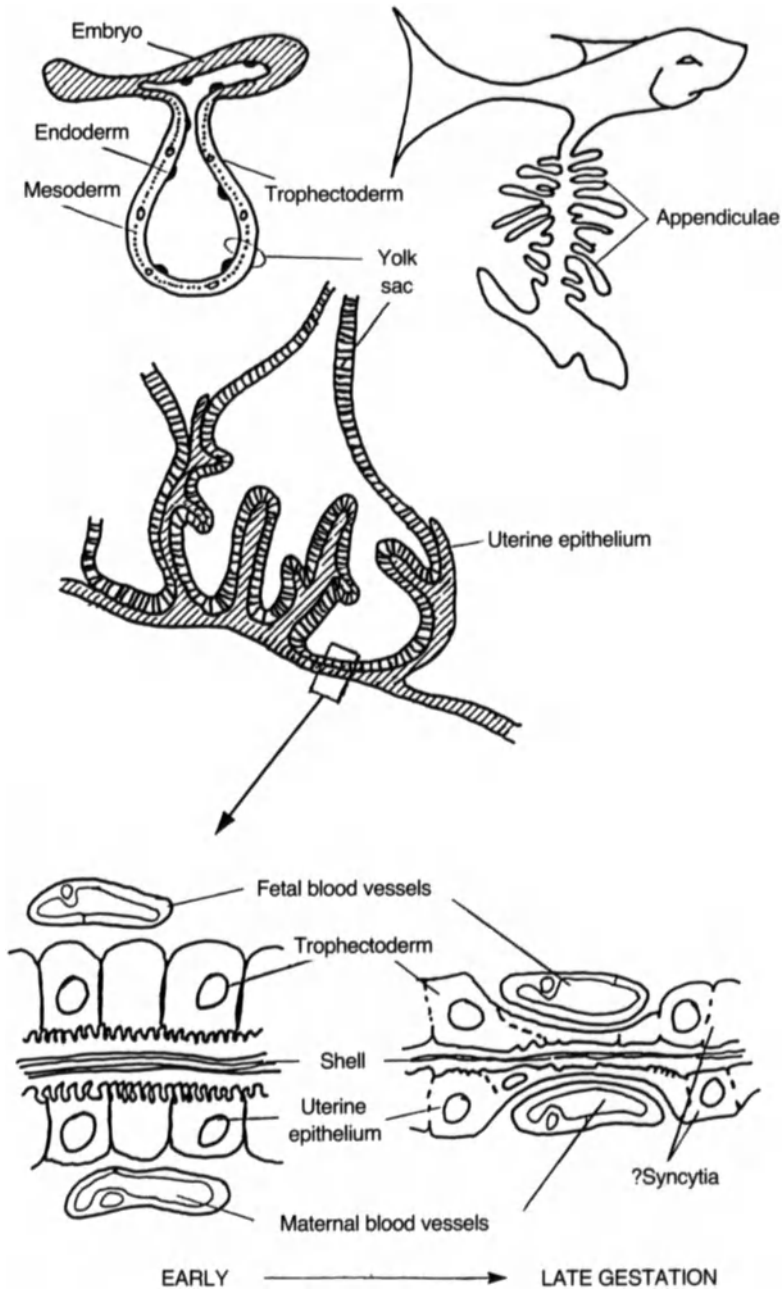


Figure 4.14 Placental development in the cartilaginous fishes; skates, rays and sharks (Chondrichthyes).

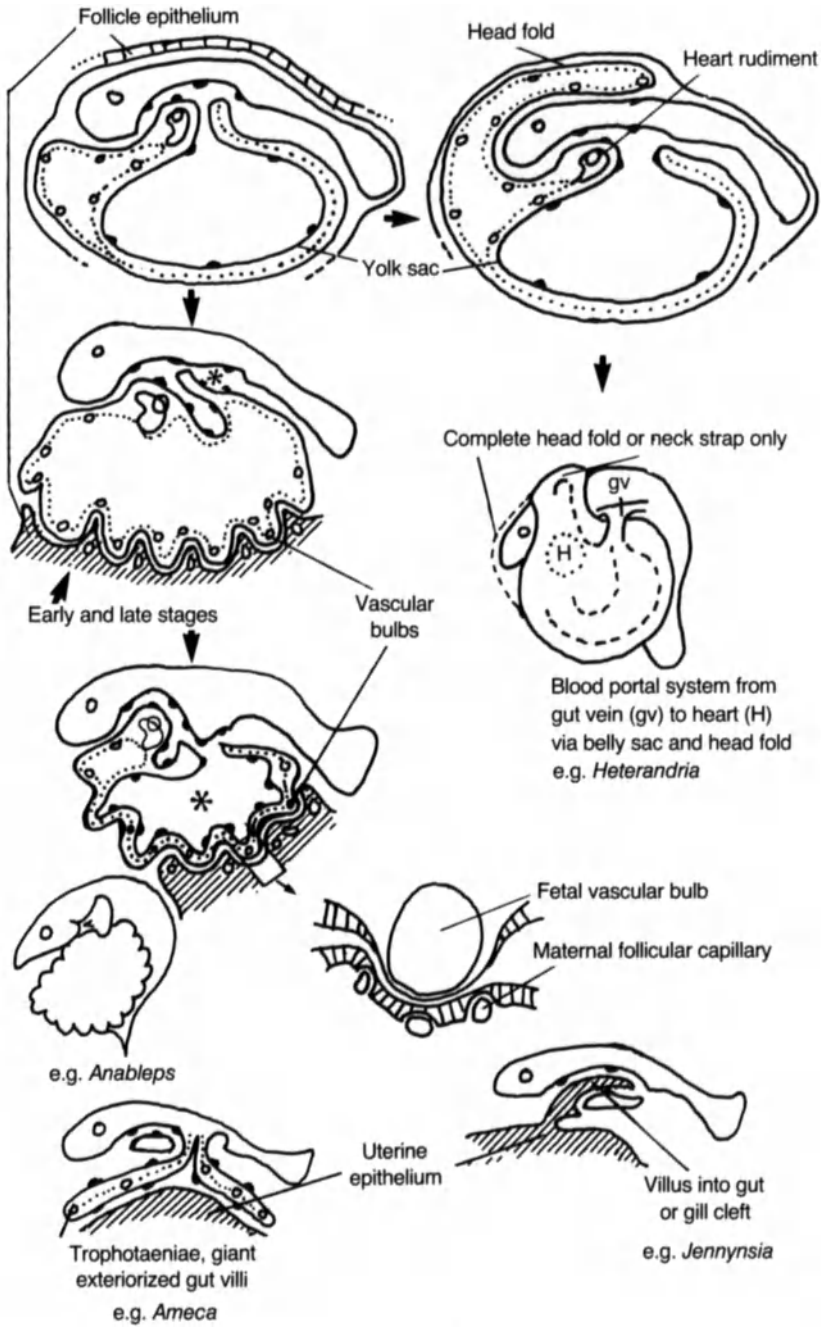


Figure 4.15 Placental membrane relationships in the bony fishes (Osteichthyes).

extraembryonic sac whose wall consists of somatopleuric mesoderm bounded by trophectoderm. This enlarges to a variable degree in different genera enclosing the entire head in some cases (Figure 4.15). The sac is richly vascularized and blood from the body flows through this portal system before returning to the heart. The trophectoderm is closely apposed to a well-vascularized follicular epithelium, and capillary indentation of fetal and maternal epithelial layers is observed in some species in later gestation. The degree of development of the pericardial sac varies considerably in different genera. *Lebistes* (Kunz, 1971) and *Poecilia* (Tavolga and Rugh, 1947) still retain a large yolk sac but the development of a small pericardial sac increases the efficiency of the organic transfer (Table 4.2). *Heterandria* has a much larger pericardial sac covering the entire head and most of the body at one stage with only a small yolk sac (Fraser and Renton, 1940; Grove and Wourms, 1991) and this combination produces a considerable increase (4 000%) in organic material during gestation. In Anableps the pericardial sac does not cover the head but expands ventrally into the area normally occupied by the yolk sac (vestigial in Anableps). The pericardial sac develops vascular swellings ('bulbs') under the trophoectoderm surface which is closely apposed to a proliferation of slender, richly vascularized villi developed from the follicular epithelium (Figure 4.15). Fetal and maternal epithelia are tightly adherent and the efficiency of this true placental structure is exemplified by the vast increase in organic transfer (800 000%) during intrafollicular development (Knight *et al.*, 1985).

The Anablepidae also show considerable embryonic gut expansion in the last third of pregnancy so that the gut wall comes to lie close to the pericardial sac with its placental function (Figure 4.15). The relevance of this juxtaposition to increased fetomaternal exchange is unknown. This association of a vascularized endodermal sac (the hind gut) with the trophectoderm of the pericardial sac is directly analogous to the formation of the allantochorion in other taxa. Many other viviparous genera show similar gut expansion and direct absorption of follicular or ovarian fluid via mouth or anus has been suggested (Wourms, 1981).

Trophotaeniae

These are extensions from the hind gut epithelium (endoderm) which are richly vascularized (by sphlanchnic mesoderm) (Figure 4.15). They range in size from small rosettes to groups of ribbon-like appendages as long as the embryo.

Trophotaeniae are present in several unrelated families, but are best developed in the Goodeidae, of which *Ameca splendens* has been best investigated (Lombardi and Wourms, 1985a,b,c; Wichtrup and Greven, 1985). Embryos are released early from the follicle into the ovarian lumen; there may be a brief pericardial sac and yolk sac stage before the development of the trophotaeniae. There is a close relationship between the trophotaeniae and the ovarian epithelium but never a permanent adherence. Ultrastructural and tracer studies indicate that the trophotaenial epithelial cells are greatly specialized for endocytotic absorption (Schindler and DeVries, 1987) and the ovarian epithelial cells for secretion. The efficiency of the system is indicated by the 15 000% increase in fetal organic material during gestation.

Other systems Two other families show large dry weight increases during gestation (Wourms, 1981). Embiotocidae (20 000% increase) have spatulate fin extensions, an enlarged hind gut and lie close to an actively secreting ovarian epithelium; and *Jennynsia* (24 000% increase) has ovarian trophonemata extending into the mouth and buccal cavity (Figure 4.15). Nothing is known of the functional cytology of these ostensibly efficient associations.

Homologies between fish and amniotic placentas

Based on embryological derivation and intimacy of apposition the chondricthyean yolk sac placenta is directly homologous to the trilaminar choriovitelline placenta of mammals. Functionally there may be differences since the most specialized fish placenta appears to have a considerable respiratory function, judging by the extensive capillary indentation and attenuation of the fetal and maternal epithelia (Cate-Hoedemaker, 1933; Schlernitzauer and Gilbert, 1966). Similar modifications are reported for the persistent trilaminar yolk sac of most marsupial families (Luckett, 1976; Tyndale-Biscoe and Renfree, 1987). This contrasts sharply with eutherians, which have tall columnar trophectodermal cells over the transient trilaminar yolk sac designed more for macromolecular uptake rather than for gas exchange.

Trophotaeniae are essentially exteriorized giant gut villi, with an endodermal epithelium vascularized by splanchnic (gut) mesoderm (Figure 4.15) (Lombardi and Wourms, 1985a,b,c). They are thus homologous to the inverted fetal yolk sacs of rodents and insectivores (Figure 4.3) and like them specialized for endocytotic uptake rather than gas exchange and with a similar loose association with the maternal epithelium.

The pericardial sac membrane of Poeciliidae and Anablepidae consists of somatopleuric mesoderm underlying trophectoderm. The extension and folding of the sac over the embryonic head (Grove and Wourms, 1991) indicates that it is functionally homologous with the chorionic headfold which produces the amniotic cavity in the amniotes. In amniotes the somatopleuric chorion would not normally be vascularized until reached by the allantoic mesoderm, but in the Poeciliidae vascularization spreads from the adjacent yolk sac portal circulation as this yolk sac is superseded by the pericardial sac (Turner, 1947; Knight *et al.* 1985). In Anablepidae the modified pericardial sac and villous elaborations of the maternal follicular epithelium are firmly adherent and Wourms (1981) seems entirely justified in defining this as a true follicular epitheliochorial placenta, not a 'pseudo' placenta (Turner, 1947). It is much more efficient than a yolk sac placenta in facilitating embryonic dry weight increase and possibly represents a parallel development to the replacement of the yolk sac by the chorioallantoic placenta in mammals. How specialized the apposed epithelia of the follicular placenta are with respect to gas exchange and macromolecular uptake remains to be determined.

Clearly the fish have evolved placental structures remarkably analogous to those in the amniotes. Apart from intrauterine cannibalism, proliferation of a non-yolk sac extraembryonic membrane in intimate association with maternal tissue seems to be the most efficient solution.

4.3.2 AMPHIBIA

(a) Introduction

Like the fish, the amphibia have evolved many viviparous forms. Most provide internal nutrition by secretions of the oviduct or ovary. There is little evidence of fetal modifications for fetomaternal transfer. Some species do show expansions of a highly vascularized tail or gill extensions, but how closely these are related to maternal tissues remains to be demonstrated.

(b) Classification

Class	Amphibia
Order	Anura

This order has some remarkable adaptations to viviparity (Hogarth, 1976). *Rheobatrachus* broods the young in its stomach, digestion being suspended for the duration. In *Rhinoderma* the male incubates the young in

its vocal chord pouch. The dorsal skin modifies into individual pouches in which the young develop in *Pipa* and *Gastrotheca*; the former has an expanded vascularized tail, the latter leaf-like gill extensions wrapped around the embryo (Del Pino *et al.*, 1975). Similar modifications are seen in many free-living juvenile forms and further investigation is necessary to establish if the association with the maternal tissue is close enough to be considered placental. *Nectophrynoides* is, more conventionally, retained in the oviduct and depends on the epithelial secretions for maintenance and growth (Xavier, 1976).

Order Urodela

Nearly all the salamanders have internal fertilization, but only one of the 400 species is obligately viviparous. It relies on ovarian secretions for growth.

Order Gymnophiona (Apoda)

Only 25 of the 170 species have been investigated; half of these are viviparous supported by lipid-rich ovarian secretions with no obvious fetal modification to facilitate absorption. Some have a long gestation period, depend on yolk for the first 3 months but then develop on ovarian secretions for a further 9 months. Viviparity is normally associated with extreme conditions of altitude or desiccation.

4.3.3 AVES

The only class with no viviparous forms. All birds have the full complement of embryonic membranes in the egg with very little variation despite enormous differences of habitat and size. The massive amount of yolk in the yolk sac is the initial and continuing source of nutrition; the allantois with deeply indented capillaries is the organ of respiration within the egg (see Mossman, 1987).

4.3.4 REPTILIA

(a) Introduction

The reptilia are mostly egg-laying amniotes with a full complement of extraembryonic membranes like the birds. The adoption of viviparity may be associated with extremes of habitat and has occurred many times (Panigel, 1956; R. Shine and Bull, 1979). Many genera today have ovoviparous and viviparous species in different climatic areas (Guillette, 1982).

The viviparous groups retain the egg in the oviduct or uterus for up to nine months; the shell is reduced to an acellular proteinaceous layer (almost to vanishing point) in some, and many ovulate eggs with very little yolk. The extraembryonic membranes have developed into structures facilitating fetomaternal exchange (J.R. Stewart and Blackburn, 1988; Blackburn, 1993; Stewart, 1993) (Figure 4.16).

The yolk sac, bi- or trilaminar, is loosely associated with the secretory uterine epithelium and the trophoblast is capable of considerable endocytotic absorption. The yolk sac placenta is present from an early stage of development and may persist to term or be reduced to a vestige by the growth of the chorioallantoic placenta. The association between trophoblast and uterine epithelium in the chorioallantoic placenta varies from a simple fetomaternal cellular apposition to those forms in which fetal and maternal capillaries deeply indent the attenuated layers (Figure 4.16). The development of this potential respiratory facility occurs just prior to the period of maximum fetal growth with its vastly increased requirement for gas exchange. Oviparous species lay their eggs just before this time (Guillette, 1982).

(b) Classification

Reptilia
Orders Chelonia (turtles), Crocodilia

All species oviparous.

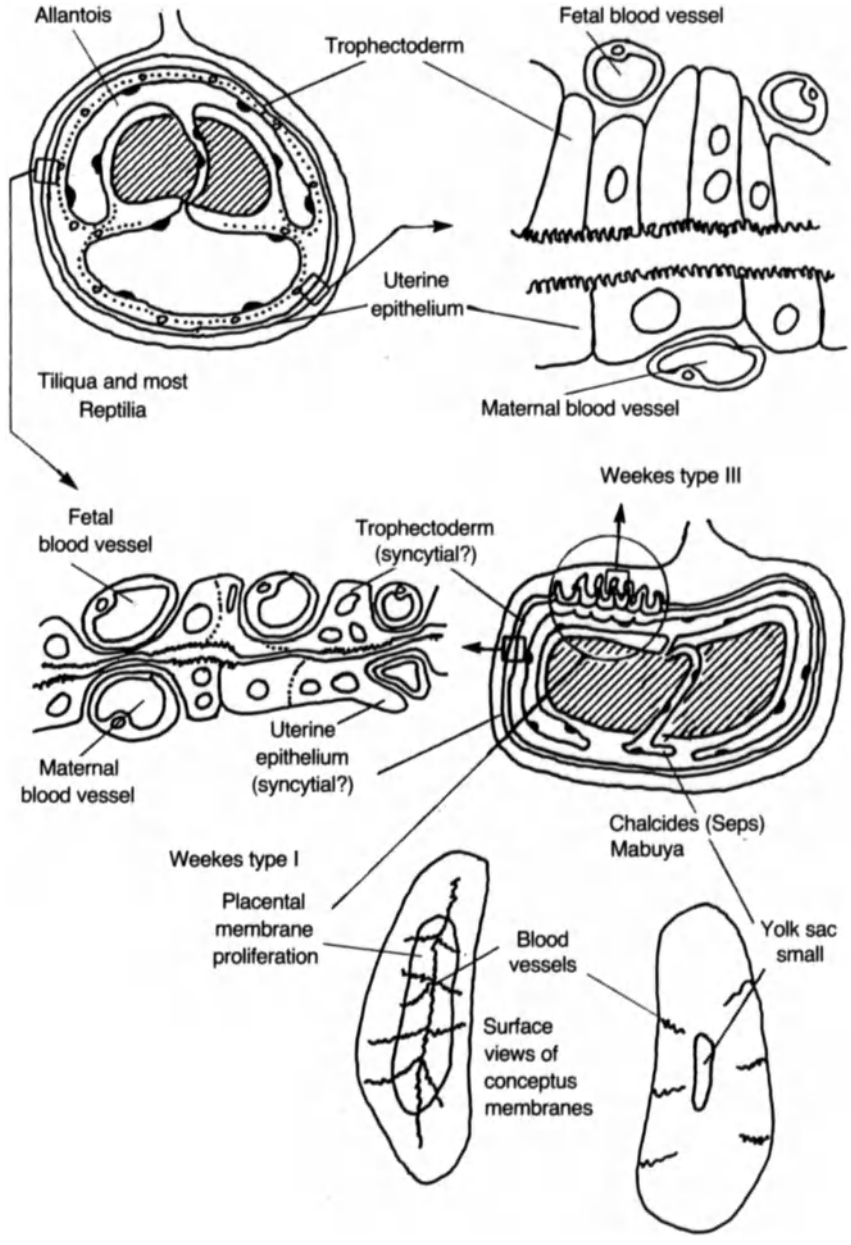


Figure 4.16 Placental membranes in reptiles. Not shown is the very thin acellular shell membrane which persists between the trophectoderm and uterine epithelium in many reptiles.

Order Squamata
Suborder Lacertiliformes (lizards)

Most families oviparous. Numerous viviparous forms (with some ovoviviparous), e.g. family Scincidae, *Lygosoma*, *Chalcides* (Seps) *Xantusia*, *Mabuya*, *Tiliqua*; Lacertidae, *Lacerta*.

Suborder Serpentiformes (snakes)

Most species viviparous.

Family Colubridae: *Thamnophis*, garter snake
Hydrophiidae: *Hydrophis*, seasnake
Viperidae: viper

(c) Suborder Lacertiliformes (lizards)

All viviparous lizards have yolk sac and chorioallantoic placentas (Bauchot, 1965: Luckett, 1977). The yolk sac is formed first and is largest in those orders with much yolk (*Tiliqua*) but may be reduced to small dimensions in those with very little (*Lygosoma*, *Chalcides*) (Figure 4.16). Initially the trophoblast is bilaminar, but it may be subsequently vascularized by yolk sac or allantoic capillaries. Both fetal and maternal layers are cuboidal, the uterine epithelium is said to be secretory, the trophoblast absorptive. Fetal yolk sac and maternal capillaries are numerous but do not indent the adjacent epithelia significantly. Fetomaternal exchange area may be increased by folding of one or both epithelia (Bauchot, 1965).

The chorioallantoic placenta is extensive in lizards, with highly vascularized fetal and maternal epithelia closely apposed in both ovoviviparous and viviparous forms. Weekes (1935) defined a range from type III with apposition of the unchanged columnar cell layers to type I in which the two epithelia are very attenuated and deeply indented by capillaries (Figure 4.16). Reports of degeneration of, or syncytial transformation in, one or both type I epithelia need to be reinvestigated with electron microscope techniques since the relevant layers may be too thin to be detected with the light microscope (Luckett, 1977). Whatever the detailed structure, the lizard chorioallantoic placenta is normally diffuse with flat apposition of fetal and maternal layers. This is the form found in the Yucca night lizard *Xantusia* in which Yaron (1977) has shown that the type I placenta is not only respiratory (with indenting capillaries) but also a major site of amino acid uptake, emphasizing the oversimplification of the respiratory as distinct from absorptive, functional classification.

However, some lizards (characteristically those with small eggs with little yolk, *Chalcides*, *Mabuya*) develop a specialized oval area in the middle of the diffuse placenta which consists of interdigitating villous folds of chorion and uterine epithelium referred to as a 'placentome' (Cate-Hoedemaker, 1933) (Figure 4.16). Unlike the remainder of the attentuated and indented (i.e. type I) fetomaternal layers the placentome in *Chalcides* has well-developed columnar epithelia (i.e. type III), presumably fulfilling the same secretory and absorptive functions as the yolk sac, which in these species is small and/or transient. A slightly different pattern is reported for *Mabuya* (Blackburn *et al.*, 1984; Blackburn, 1993), which is said to have the same 'absorptive' (type III) structural type in both placentome and at least part of the surrounding areas of the chorioallantoic placenta. Since *Mabuya* has the smallest egg and the biggest percentage increase in dry weight during gestation so far found in lizards (38 000%) it is possible that detailed investigation may yet demonstrate areas with the usual respiratory structural specialization (type I).

Fetal binucleate cells have been reported in the chorion of the placentome in both *Mabuya* and *Chalcides*. There is no evidence for migration or any significant differences in cytoplasmic structures with adjacent uninucleate cells. This is a further example of the variety of form shown by the trophoblast, but there is no evidence for any specialized role such as that demonstrated for the equid (Allen, 1982)

or the ruminant binucleate cell (Wooding, 1982b).

(d) Suborder Serpentiformes (snakes)

The placental structures of viviparous snakes are similar to those of lizards. However, several forms show the modification of an early absorptive yolk sac placenta into a unique, discoid chorio-omphaloallantoic placenta (Hoffman, 1970; J.R. Stewart and Blackburn, 1988; Stewart, 1993). This is complemented or replaced by a later chorioallantoic placenta frequently showing adaptations facilitating respiratory exchange. No specialized placentomal areas have been reported. Hoffman (1970) has produced autoradiographic evidence for the yolk sac chorion as a major site of nutrient uptake. In *Thamnophis* (Hoffman, 1970) and *Hydrophis* (Kasturirangan, 1952) chorionic binucleate cells are present in the yolk sac region instead of the chorioallantoic placenta as in lizards but with a similar absence of specialized structure.

(e) Homologies between reptile and amniotic placentas

The reptiles thus show many examples of the development of specialized fetomaternal extraembryonic membrane apposition as a result of reduction or loss of the shell membrane in eggs retained in the uterus.

The yolk sac is important in many species, providing both respiratory and nutrient uptake. It is never as specialized as in some elasmobranchs (*Mustelus canis*) for respiratory exchange because this function is provided by the chorioallantoic placenta and the two placentas coexist in snakes and many lizards.

In the most highly developed lizards with the smallest, least yolky eggs and earliest shell loss, the chorioallantoic placenta supersedes the early yolk sac and develops two structurally different areas. A central region is elaborately folded, increasing the surface area available for endocytotic uptake, and the remainder is specialized for respiratory exchange. This arrangement is functionally analogous, but structurally reversed, to that seen in the mammalian chorioallantoic placenta, in which the folded area provides for respiration.

In the reptiles this increase in extent and specialization correlates with the exponential growth phase of the embryo and seems to be essential to supply sufficient nutrient and gas exchange to ensure a successful uterine development.

4.3.5 CLASS MAMMALIA, SUBCLASSES PROTOTHERIA (MONOTREMATA, e.g. PLATYPUS) AND METATHERIA (MARSUPIALIA)

(a) Introduction

Prototheria lay eggs and Metatheria produce shelled eggs *in utero* with the same reliance as birds on a bi- or trilaminar yolk sac and allantois for mobilization of yolk and respiratory exchange. However, they all have far less yolk and a much earlier hatching or birth than the birds and require subsequent mammary nutrition and protection in a pouch in Metatheria (Luckett, 1976, 1977; Tyndale-Biscoe and Renfree, 1987). A few marsupials lose their shell and show localized invasion of the uterine epithelium by the yolk sac trophectoderm, and other families develop an area of chorioallantoic placentation in the last quarter of the typically very short 12–14 day gestation.

(b) Prototheria (Monotremata)

Two families only

Ornithorhyncus: Platypus
Tachyglossus: Echidna

The eggs are much smaller than those of equivalent sized reptiles or birds with a much greater increase in size by absorption of uterine secretions *in utero* prior to shell depo-

sition. The blastocyst forms precociously and has 19 somites when the egg is laid. The mesoderm has spread to produce a vascularized trilaminar yolk sac over two-thirds of the blastocyst with the remainder bilaminar and avascular (Luckett, 1977) (Figure 4.17).

The trophectoderm of the trilaminar region is thin, minimizing the gas diffusion distance to the yolk sac capillaries, while the endodermal cells are columnar, actively absorbing yolk and transporting it to the same capillaries. In the avascular bilaminar portion the cell types are reversed, a columnar absorptive ectoderm and thin endoderm.

After the egg is laid the amnion folds fuse and the allantois develops as the usual hind gut outgrowth eventually forming a chorioallantoic membrane over half the inside of the egg. The trilaminar yolk sac persists over the other half; the bilaminar segment is finally a mere remnant (Luckett, 1977) (Figure 4.17).

The trophectoderm over the late-gestation trilaminar yolk sac and chorioallantois is said to be thin, which would facilitate haemotrophic exchange, but there are no detailed studies available to verify this.

(c) Metatheria (Marsupialia)

Seven major families

Dasyuridae: insectivores and carnivores
Didelphidae: American opossums
Peramelidae: bandicoots
Macropodidae: kangaroos, wallabies
Phalangeridae: arboreal Australian opossums (*Trichosurus*)
Phascolarctidae: koala
Vombatidae: wombats

There is an excellent recent review of marsupial reproduction (Tyndale-Biscoe and Renfree, 1987, and Chapter 7, this volume).

Metatheria have even less yolk than Prototheria, cleavage is complete (holoblastic) and the hollow blastocyst is retained in the uterus initially bounded by a shell membrane.

Pregnancy is completed within the duration of one ovarian cycle; there is no system for prolonging the life of the corpus luteum.

Most families have similar fetal membrane development to Prototheria, but the allantois is smaller and forms a chorioallantoic placenta in only a few species (Figure 4.17).

The bilaminar yolk sac persists to the end of pregnancy absorbing uterine secretions by endocytosis before and after shell membrane rupture. Amino acids are taken up by this route and the yolk sac lumen can contain up to 10 times the concentration in the maternal plasma. The trilaminar yolk sac occupies as much as two-thirds of the egg surface and shows a thin trophectodermal layer over the yolk sac capillaries, suggesting a predominantly respiratory function.

In most families the trophectoderm of the bi- or trilaminar yolk sac is loosely apposed to the uterine epithelium. After the shell membrane disappears in mid- to late pregnancy some species show a trophectodermal invasion of the uterine epithelium from a discontinuous annular zone close to the junction of bi- and trilaminar portions of the yolk sac. The invasive trophectodermal process may be a syncytium (Didelphidae, *Philander*) (Enders and Enders, 1969) or cellular (Dasyuridae; *Sminthopsis*) (Figure 4.17) but normally does not cross the uterine epithelium basement membrane although there is one report that it surrounds superficial maternal blood vessels (Tyndale-Biscoe and Renfree, 1987). There are interesting parallels here with the initial stages of girdle formation in the horse (section 4.3.6.a).

The allantois is reported to form a chorioallantoic placenta in only three families, Phascolarctidae, Vombatidae and Peramelidae, and only the last has been investigated in detail. In the peramelids, *Perameles* and *Isoodon*, the allantois vascularizes the trophectoderm in the last one-third of a very short 12-day pregnancy (Padykula and Taylor, 1976). It forms a chorioallantoic placenta in close apposition to the uterine epithelium

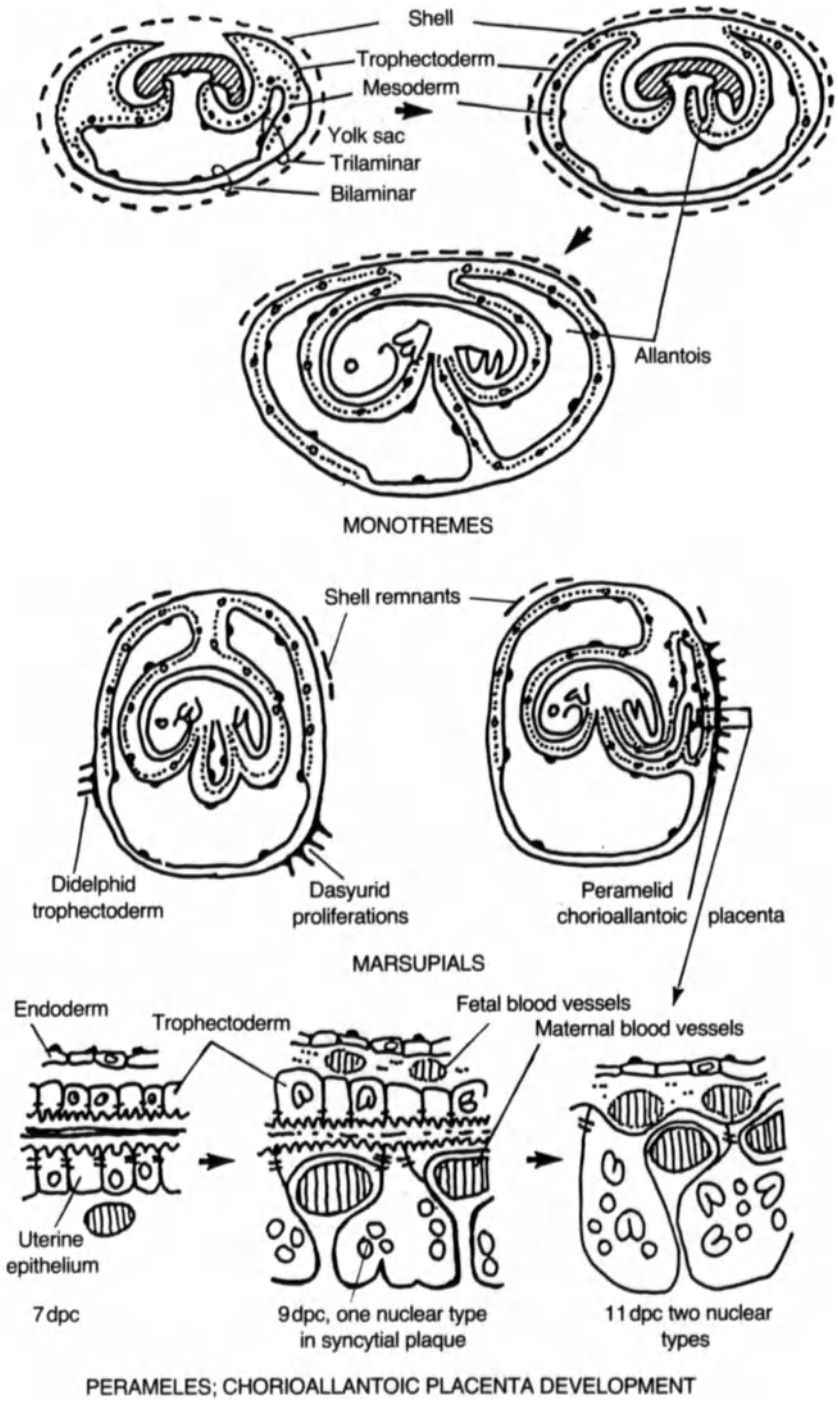


Figure 4.17 Placental membranes in monotremes and marsupials.

occupying a far smaller area than the persistent bi- and trilaminar yolk sac placentas (Figure 4.17). In *Perameles* at 9 days post coitum or post ovulation, the entire uterine epithelium apposed to the conceptus develops from a unicellular layer into individual plaques of syncytium containing uniformly small round nuclei. The uterine capillaries deeply indent these syncytial plaques. The apposed trophectoderm is undifferentiated and unicellular with large irregularly shaped nuclei. As the vascularization of the chorioallantoic trophectoderm increases, the fetal blood vessels indent the epithelium and now two sorts of nuclei are recognizable in the uterine epithelial syncytial plaques, small round ones and others with large irregular profiles. Eventually the unicellular trophectoderm can no longer be distinguished and only very thin laminae continuous with the syncytial plaques separate maternal and fetal blood vessels (Padykula and Taylor, 1976) (Figures 4.17, 4.18 and 4.19).

From the time course of the trophectodermal disappearance and the evidence of the two types of nuclei in the syncytial plaques it seems that the unicellular trophectoderm cells fuse with the uterine epithelial syncytial plaques. Such fetomaternal cell fusion would significantly reduce the number of membrane barriers between fetal and maternal blood and greatly facilitate fetomaternal exchange. The trophectoderm does not vanish but forms part of a fetomaternal hybrid syncytium directly analogous to the similar system produced in sheep and goat chorioallantoic placentas by fusion of fetal binucleate cells with uterine epithelial cells (section 4.3.6.b).

The chorioallantoic placenta in the peramelids supports considerable embryonic growth. Even though their gestation is the shortest of any marsupial, their neonates are relatively well developed at birth (Tyndale-Biscoe and Renfree, 1987). This indicates that the chorioallantoic placenta may well be more efficient than the yolk sac type.

The marsupials thus show several features, such as trophectodermal invasion and formation of chorioallantoic fetomaternal hybrid tissue, which have close parallels in certain eutherian placentas. This seems to be another example of parallel evolution, the same functional demands producing similar structures from basically the same fetal membrane repertoire, rather than any indication of an evolutionary relationship between the groups.

4.3.6 EUTHERIA

(a) Epitheliochorial placentation (pig, horse)

In this form of placentation the uterine epithelium and trophectoderm come into contact, the fetal and maternal microvilli interdigitate and placental development essentially involves a vast increase in area with no loss of any layers between the fetal and maternal bloodstreams. The exclusively cellular layers may become exceedingly attenuated, reducing the diffusion distance as pregnancy proceeds, but all persist to term (Amoroso, 1952; Steven 1975a, 1983; Ramsey, 1982; Mossman, 1987).

The uterine glands are numerous and actively secreting through pregnancy (Bazer and First, 1983), and there are fetal expansions over the gland mouths, forming areolae consisting of cells specialized for histotrophic absorption. The domestic pig placenta will be described as the basic type, and variations on that pattern considered for the horse, American mole (*Scalopus*) and the primates (lemurs and *Galago*).

Pig, *Sus scrofa*

Oestrous cycle: 21 days

Ovulation: spontaneous
*Uterus: bicornuate
Litter: 6–12

Gestation: 114 days
*Implantation: superficial
*Amniogenesis: folding
*Yolk sac: initially large and vascularized, remnant persists, apparently functional to 50 dpc
*Chorioallantois: forms definitive placenta
*Shape: diffuse, simple villous
*No decidual reaction
*Interhaemal membrane: epitheliochorial
Accessory placental structures: areolae (Brambel, 1933; Friess *et al.*, 1981)

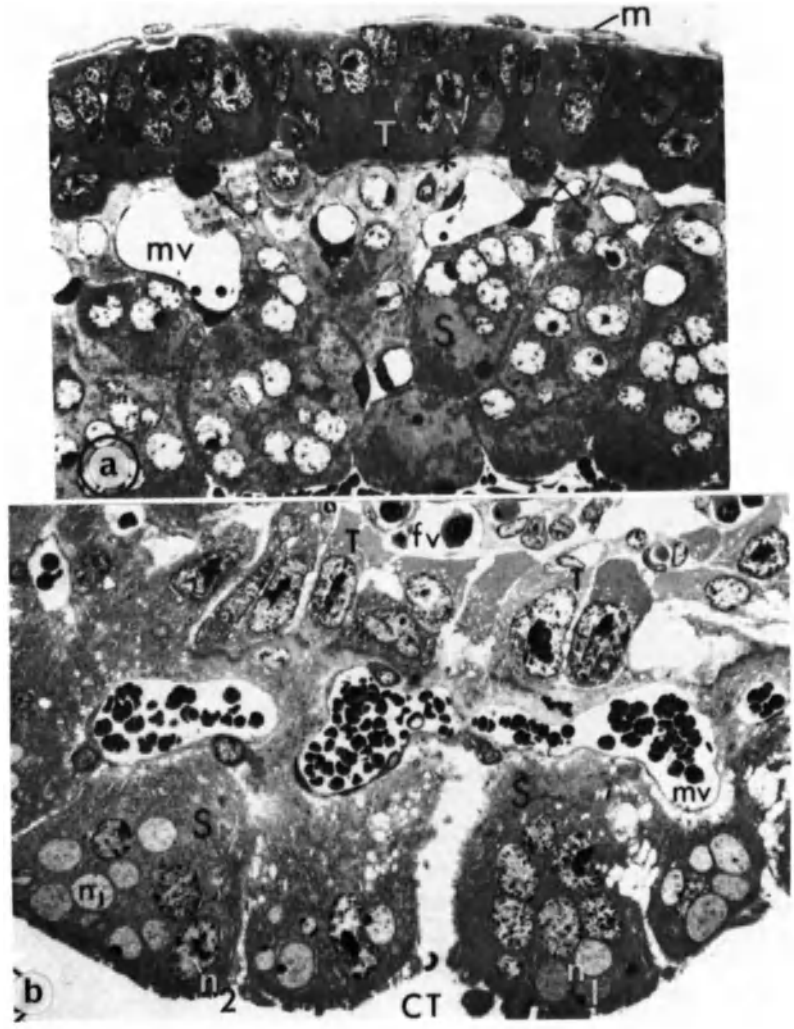


Figure 4.18 Development of chorioallantoic placenta in marsupials (compare with bottom of Figure 4.17). (a) 7 mm crown–rump (CR) length; close apposition (*) between the cellular fetal trophectoderm (T) (m, fetal mesoderm) and uterine epithelial syncytial plaques (S). Note that the plaques are deeply indented by the maternal capillaries (mv). *Isoodon*, × 400. (b) 10 mm CR. The fetal blood vessels (fv) do not indent the cellular fetal trophectoderm (T) in the way that the maternal vessels (MV) indent the uterine epithelial syncytial plaques (S). The plaques contain two sorts of nuclei: n_2, morphologically similar to those in the trophectoderm cells; and n_1, similar to those in the original syncytial plaques. CT maternal connective tissue. *Perameles*, × 400.

The major characteristics (*) are found in the families Suidae, Hippopotamidae and Camelidae (Mossman, 1987).

Pig fetal membranes At implantation (15 dpc, see below) the conceptus is a mostly trilaminar blastocyst. The yolk sac forms what is probably a briefly functional bilaminar absorptive yolk sac placenta before its attachment to the trophectoderm is disrupted by growth of the allantois. The yolk sac lumen then collapses but still retains a good blood supply. Healthy endodermal cells with enormous mitochondria and large amounts of smooth endoplasmic reticulum are found in the yolk sac up to 50 days of gestation (Tiedemann and Minuth, 1980).

Between 22 and 28 dpc the allantois expands to contact and vascularize most of the inner surface of the trophectoderm. This forms the chorioallantoic placenta and the vascularization also spreads into the amniochorionic membrane over the back of the embryo (Amoroso, 1952) (Figure 4.1).

Development of the pig placenta At 8–11 dpc the pig conceptuses are flaccid spheres reaching 10 mm in diameter and have been spaced evenly throughout the uterus probably by contractions of the uterine musculature (section 4.2.9a). Between 10 and 12 dpc the conceptuses secrete oestrogens (Geisert *et al.*, 1982a; Bazer *et al.*, 1984, 1989) and an interferon-like molecule between 11 and 17 dpc (Cross and Roberts, 1989). A minimum of four conceptuses are necessary to stimulate the uterus to modify its prostaglandin secretion so that the luteal progester-

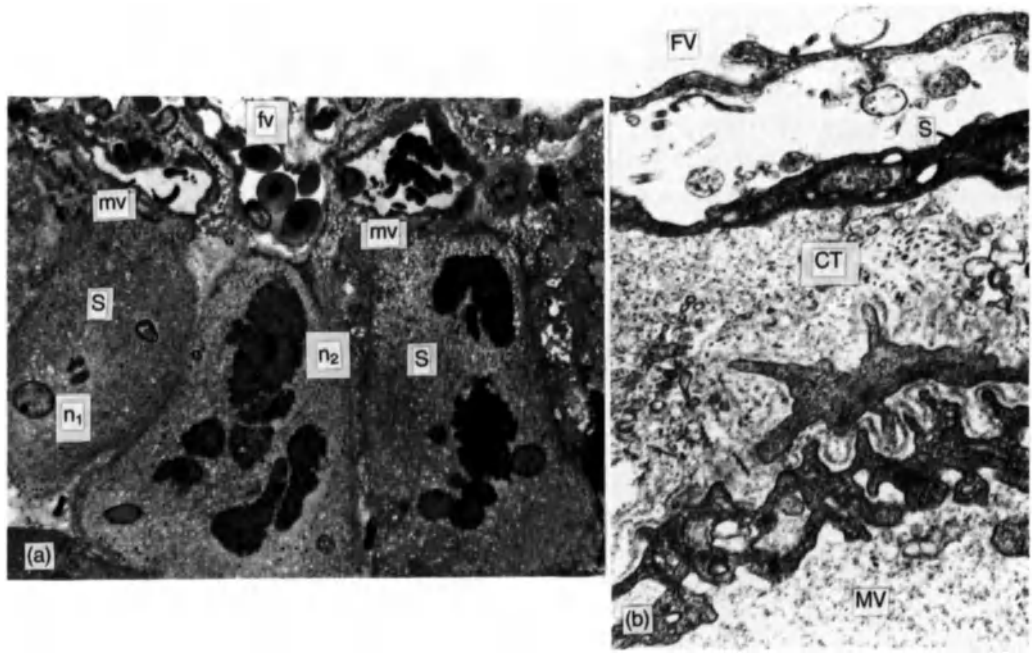


Figure 4.19 Definitive structure of the chorioallantoic placenta in marsupials. (a) Near term, 12 mm CR. The fetal trophectoderm is no longer visible but the fetal blood vessels (fv) are separated from the maternal (mv) by only a thin layer of cytoplasm continuous with the syncytial plaques (S), which contain two sorts of nuclei (n_1, n_2). *Isoodon*, × 480. (b) 12 mm CR. Electron micrograph of the attenuated cytoplasmic layer (S) between fetal (FV) and maternal (MV) blood vessels. CT, maternal connective tissue. *Isoodon*, × 15 000. (From Padykula and Taylor, 1976.)

one production is maintained. Subsequent fetal death may result in litters of less than four (Polge *et al.*, 1966). There is also evidence for an optimal length of uterus per conceptus at 20–25 dpc (M.C. Wu *et al.*, 1989).

The oestrogens also stimulate secretion from the uterine epithelium and glands (Geisert *et al.*, 1982b). This produces specific proteins which are thought to be involved in the remarkable reorganization and subsequent immunological readjustments necessary for successful implantation of the conceptus and continued luteal growth (Roberts *et al.*, 1985; Bazer *et al.*, 1989). Similar proteins are produced by the uterus of a non-pregnant pig treated at the correct stage of the cycle with artificial oestrogens (Geisert *et al.*, 1982c).

The 10 mm spherical conceptus extends in about 24 h to become a 150-mm thread stretched along the considerably corrugated uterine epithelium (Figure 4.20a) (Perry and Rowlands, 1962).

No DNA or RNA synthesis or any mitoses occur during this structural remodelling (Geisert *et al.*, 1982b; Mattson *et al.*, 1990). Once elongated, the conceptus thread interacts with the immediately underlying uterine epithelium. From this time (12–13 dpc) marked changes throughout the uterus in the structure of the subepithelial capillaries, notably increased fenestrations, have been described (Keys and King, 1988) and assumed to indicate increased permeability. However, the local increase in permeability in the immediate vicinity of the blastocyst reported by Keys *et al.* (1986) could not be verified by subsequent work (Keys *et al.*, 1989). On 13–15 dpc the fetal and maternal epithelia come into close apposition, involving loss of microvilli and considerable glycocalyx erosion, along the strip region (Dantzer, 1985). As the fetal and maternal microvilli reform they now interdigitate. This increases the fetomaternal exchange area by 10–12 times over a flat apposition (Baur, 1977). There is no good evidence for formation of any junctions between the fetal and maternal epithelia or for penetration of their tight junction apical seals by cellular processes from either side. Neither epithelium forms even a limited syncytium at any stage of gestation (Dantzer, 1985). There are few differences in the fine structure of the uterine epithelium between the cycle and early pregnancy (Keys and King 1989, 1990). Since fetal trophectodermal syncytium formation has been reported from ectopic pig blastocysts transferred into the pregnant endometrium below the uterine epithelium (Samuel and Perry, 1972), this layer may be instrumental in blocking fetal syncytium formation and possible subsequent invasion. No decidual changes have been reported in the endometrium at the implantation site.

At 17 dpc proliferation of fetal trophectoderm over the gland mouths has started to form the regular areolae, where the fetal and maternal epithelia become widely separated by glandular secretion (Amoroso, 1952) (Figures 4.20b,c and 4.21a).

About 7000 regular areolae per conceptus are present, fairly evenly spaced, from early gestation to term (Figure 4.20c). They triple in area between 30 and 114 dpc forming 10% of the total chorioallantoic surface area at midgestation and 4% at term (Brambel, 1933). They show a characteristic fine structure (Sinowatz and Friess, 1983) and a broad spectrum of enzyme activities (Skolek-Winnisch *et al.*, 1985; Firth *et al.*, 1986a,b). Uteroferrin, an iron-containing glycoprotein, is among a variety of molecules synthesized in the gland cells (Chen *et al.*, 1975; Stroband *et al.*, 1986). This is released into the lumen, taken up by the areolar trophectodermal cells and transferred to the fetus, for which it represents an important iron source (Raub *et al.*, 1985). The larger and less frequent (about 1500 per placenta) irregular areolae have significantly different fine structure from the regular type, but nothing is known of their specific functions (Figure 4.21) (Brambel, 1933; Perry, 1981).

At 18–22 dpc the conceptus expands so that it touches the uterine epithelium round

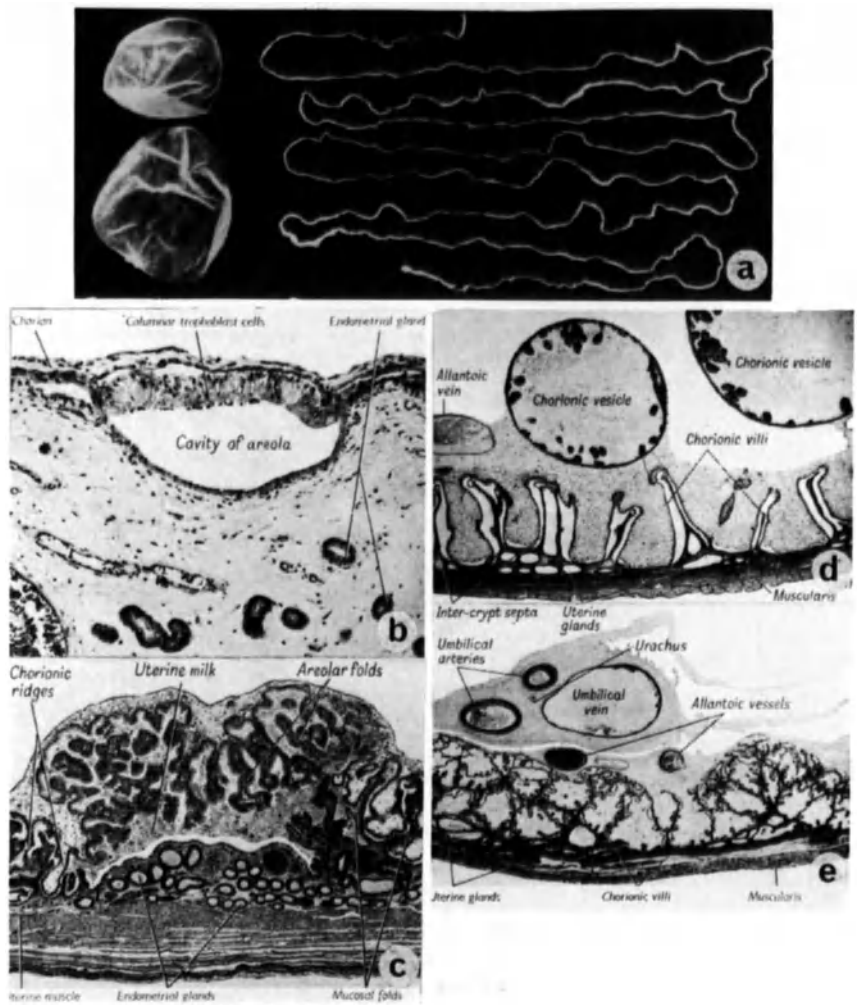


Figure 4.20 Epitheliochorial placentation. (a) Note the remarkable change of the shape of the pig blastocyst from a 5-mm-diameter sphere at 10 dpc to a 1-m-long thread at 13 dpc, × 0.5. (From Perry and Rowlands, 1962.) (b and c) Development of a regular areola in a pig placenta. (b) 23 dpc, × 80. (c) mature, 111 dpc, × 25. (d and e) Section through mature lemuroid placentas. (d) *Loris*, × 15. (e) *Galago*, × 7. Compare with the pig placenta in Figure 4.21.

the entire circumference and establishes microvillar interdigitation over the whole area of the trophectoderm (Dantzer, 1985; Hasselager, 1985). Further growth in the area of the fetomaternal junction is accomplished by development first of folds and then division of these into simple villi (K. Michael *et al.*, 1985; Wigmore and Stickland, 1985) (Figure 4.22).

The result is to maximize the surface area available for exchange between the fetal and maternal capillaries and minimize the distance between them. This is achieved in the pig by development of a meshwork of capillaries just under the epithelium of each fetal and maternal villus. Injection of latex or resin into the fetal or maternal vasculature and examination of the resulting casts of the

blood vessel architecture shows that the fetal and maternal villus meshworks are complementary and each is supplied by an artery running up one side of the villus before dividing near the apex into a capillary meshwork which drains to veins around the base of the villus (Figures 4.9, 4.23 and 4.24) (Tsutsumi, 1962; MacDonald, 1975, 1981; Leiser and Dantzer, 1988) (for the physiological exchange implications of this arrangement see section 4.2.5c).

Increase in size and branching of the villi can be accommodated by expansion of the capillary meshwork. As the conceptus grows the fetomaternal junctional area and the transport capacity of the placenta increase continuously right up to term (K. Michael *et al.*, 1985). The villi and folds show local differentiation of structure. The trophectodermal cells at the bases of the fetal villi are tall, columnar and specialized for histotroph absorption; they are apposed to similar columnar maternal uterine epithelial cells, often with cell debris between (Dantzer *et al.*, 1981). The fetal and maternal blood vessels are situated just below the basement membranes. The structure is analogous to a miniareola with material produced by degeneration of, or secretion by, the maternal epithelium rather than a gland. In contrast, at the sides and tips of the fetal villi (sides and base of the maternal villi) both epithelia are thin and deeply indented by blood vessels from each side (Figures 4.22b and 4.25) (Steven, 1983).

This reduces the interhaemal diffusion distance to as little as 2 μm, although the same number of membrane barriers is present as at

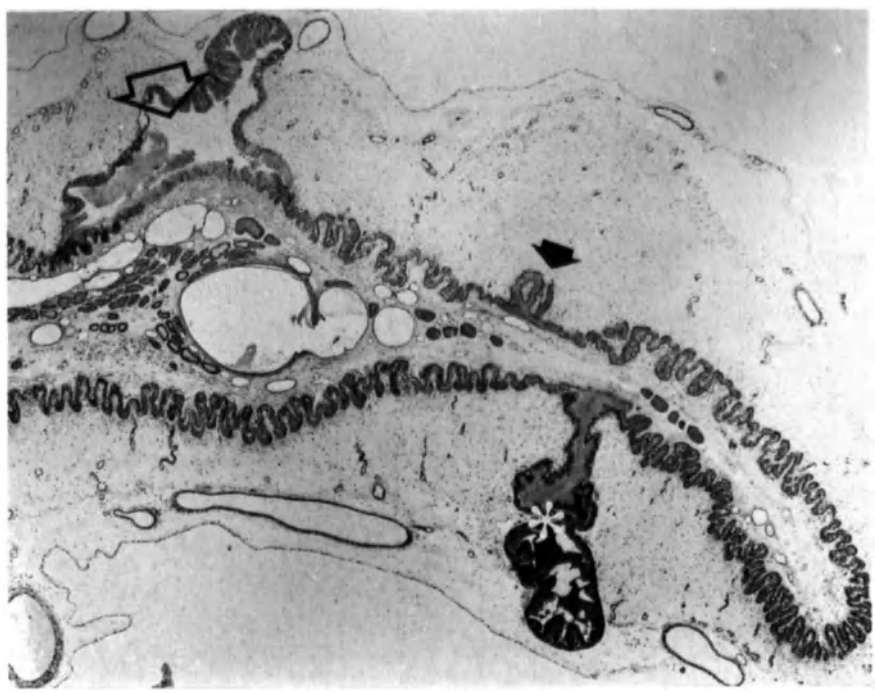


Figure 4.21 Epitheliochorial placenta of the pig. Light micrograph showing a maternal mucosal fold whose sides consist of fetal villi inserted into endometrial crypts, seen at higher magnification in Figure 4.22b. A chorionic vesicle (asterisk) and irregular (open arrow) and regular (closed arrow) areolae can be seen. The section passes through the extreme edge of the regular areola, whose characteristic structure is better illustrated on Figure 4.20c. 107 dpc, × 12.

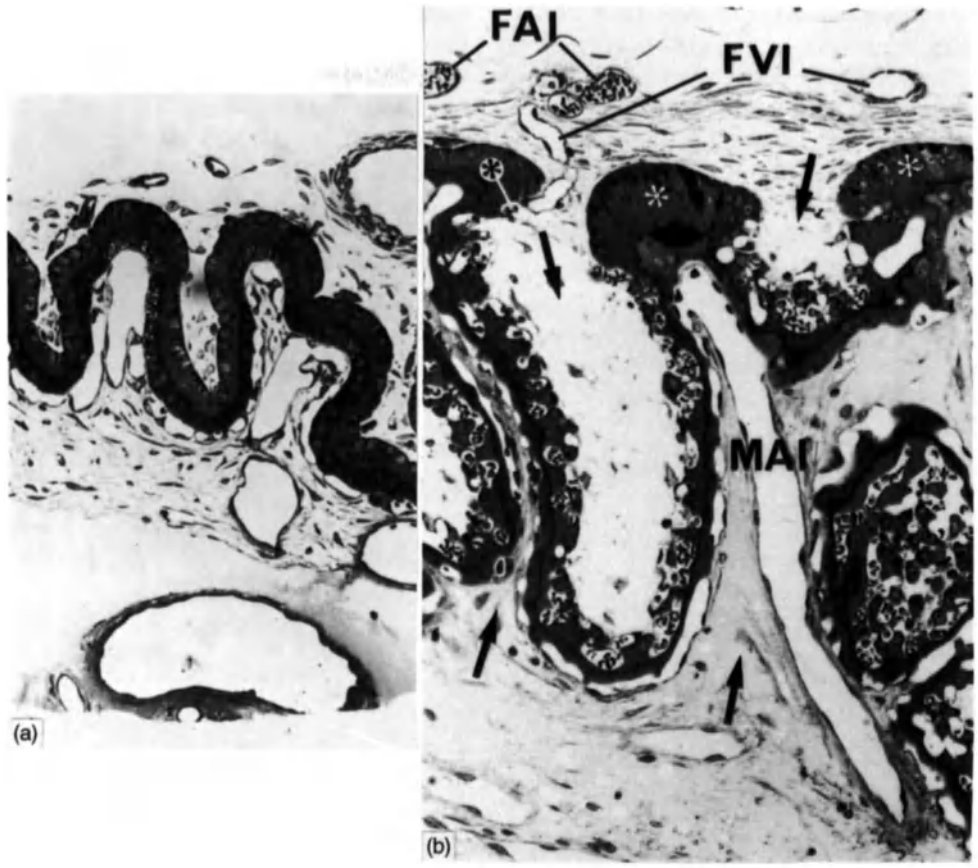


Figure 4.22 Epitheliochorial placenta of the pig. Development of the placental villi which form the fetomaternal haemotrophic exchange area. Note the increase in regional differentiation. The uniform trophectoderm and uterine epithelial cell layers forming the folds of (a) develop into very attenuated layers deeply indented by fetal and maternal capillaries around the apex and sides of the fetal villus in (b). However, at the base of the fetal villi in (b) the trophectoderm cells are considerably enlarged (asterisks) and are involved in pinocytotic, histotrophic uptake. Upward arrows, sides of maternal crypts; downward arrows, fetal villi; FAI fetal arteriole; FVI fetal venule; MAI maternal arteriole. (a) 33 dpc, × 130. (b) 99 dpc, × 170.

the base of the fetal villi where the interhaemal distance ranges from 20 to 100 μm (Figures 4.22 and 4.25).

Indentation of the blood vessels into the epithelia increases as pregnancy proceeds (Figures 4.22 and 4.25). There is no evidence for any active process of 'tunnelling into' or 'invasion' of the epithelia by the blood vessels. The endothelium is always separated from the epithelium by a basement membrane. It seems likely that indentation results from differential growth, the endothelium growing less quickly than the epithelium.

During pregnancy the fetal and maternal villi can be separated manually but there is always some maternal epithelium pulled away with the fetal side and vice versa. At parturition the placenta separates cleanly at the microvillar fetomaternal junction and the incidence of retained placentas (see synepith-

eliochorial placentation) is negligible. There is a massive release of relaxin from the corpora lutea as a result of the rise in prostaglandins which triggers parturition (First and Bosc, 1979). Processes initiated by these hormonal changes (Bazer and First, 1983) are no doubt involved in releasing the microvillar interdigitation between fetal and maternal epithelia to allow an easy placental separation.

Horse, Equus caballus

Oestrous cycle: 22 days

*Ovulation: spontaneous
*Uterus: bicornuate
Gestation: 336 (± 20) days
Litter: one, rarely two (Ginther, 1989)
*Implantation: superficial
*Amniogenesis: folding
*Yolk sac: initially large and functional to 38 dpc
*Chorioallantois: forms definitive placenta
*Shape: diffuse, microcotyledonary, villous
*No decidual reaction.
*Interhaemal membrane: epitheliochorial
Accessory placental structures: trophectodermal girdle producing endometrial cups; areolae

The major characteristics* are found in the Perissodactyla families.

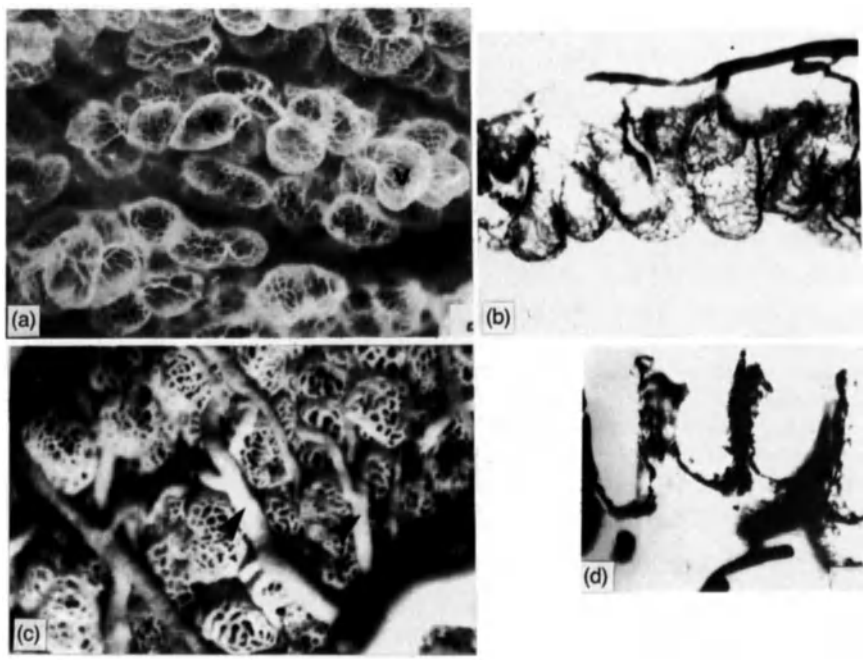


Figure 4.23 Epitheliochorial placentation. Pig placenta vascular supply demonstrated with corrosion casts (a and c) and cleared sections of injected specimens (b and d). See also Figures 4.8, 4.9 and 4.24. (a) Latex injection of fetal blood supply viewed from the maternal side showing the capillary network in each villus (v). 105 dpc, × 40. (b) Injection of the fetal blood supply shows the villus capillary net in cross section. 105 dpc, × 40. (c) Latex injection of the maternal vasculature viewed from the back showing the network of capillaries at the base of each crypt with their supplying or draining arterioles and venules (arrowheads). 105 dpc, × 40. (d) Injection of the maternal blood supply demonstrates on a section the maternal crypts into which the fetal villi fit. 75 dpc, × 50. (From Tsutsumi, 1962.)

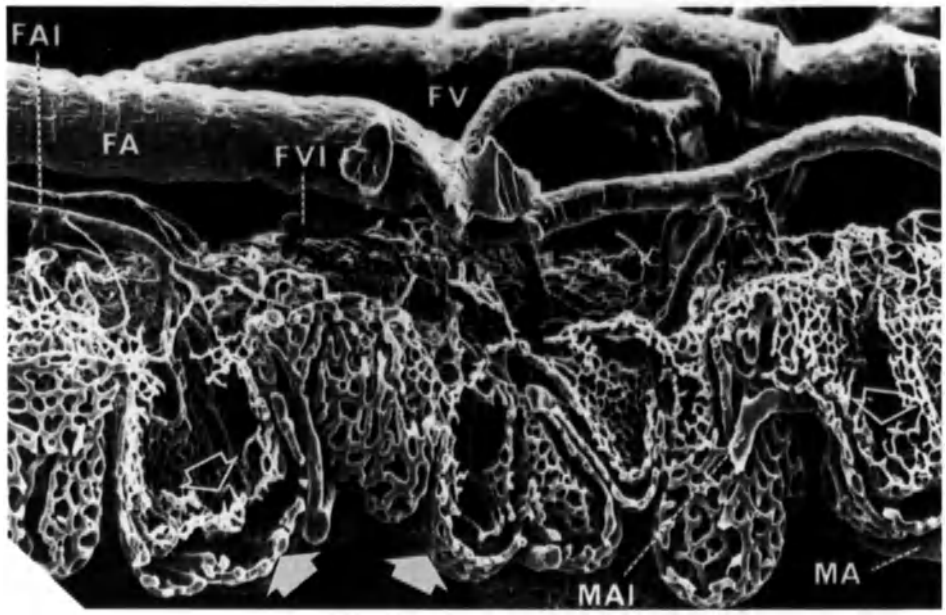


Figure 4.24 Epitheliochorial placentation. Scanning electron micrograph of a corrosion cast from a pig placenta with both maternal and fetal circulations injected with acrylic resin *in situ* and then cross-fractured. The complementary nature of the fetal villus (white open arrows) and maternal crypt (solid white arrows) capillary networks is elegantly demonstrated. FAI, fetal arteriole; FVI, fetal venule; FA, fetal artery; FV, fetal vein; MAI maternal arteriole; MA, maternal artery. 99 dpc, × 95. (From Leiser and Dantzer, 1988.)

Equidae, horse, donkey, zebra
Tapiridae
Rhinocerotidae

The unusual length of standing heat in horses (4–5 days; compare with sheep, 1 day; pig, 1½ days) and the consequent uncertainty about the time of ovulation makes days post coitum a far less reliable indicator of gestational age than in the other domestic species. Use of the more accurate days post conception is only realistic if the preovulatory surge in luteinizing hormone is monitored in the maternal blood for a particular pregnancy.

Like the pig, the definitive horse placenta is a simple apposition of unchanged fetal and maternal epithelia for haemotrophic exchange with a large number of areolae for histrophic transfer (Steven, 1982). Unlike the pig, the horse conceptus does not elongate and implantation (fetomaternal microvillar interdigitation) occurs much later at about 45 dpc (pig 15 dpc) (Ginther, 1979). Uniquely, the equid trophectoderm produces (between 30 and 40 dpc) a girdle around the conceptus of binucleate cells which migrate into the maternal endometrial stroma to form the gonadotrophin-producing endometrial cups (Allen *et al.*, 1973; Allen, 1982). This pregnant mare serum gonadotropin (PMSG) is now known as equine chorionic gonadotropin (eCG).

Horse fetal membranes At 20 dpc the 30–40-mm spherical conceptus has a fully formed amnion and the allantois is just visible as a small outgrowth of the hind gut (Figure 4.26a).

About one-third of the conceptus is bilaminar and most of the rest forms a rapidly

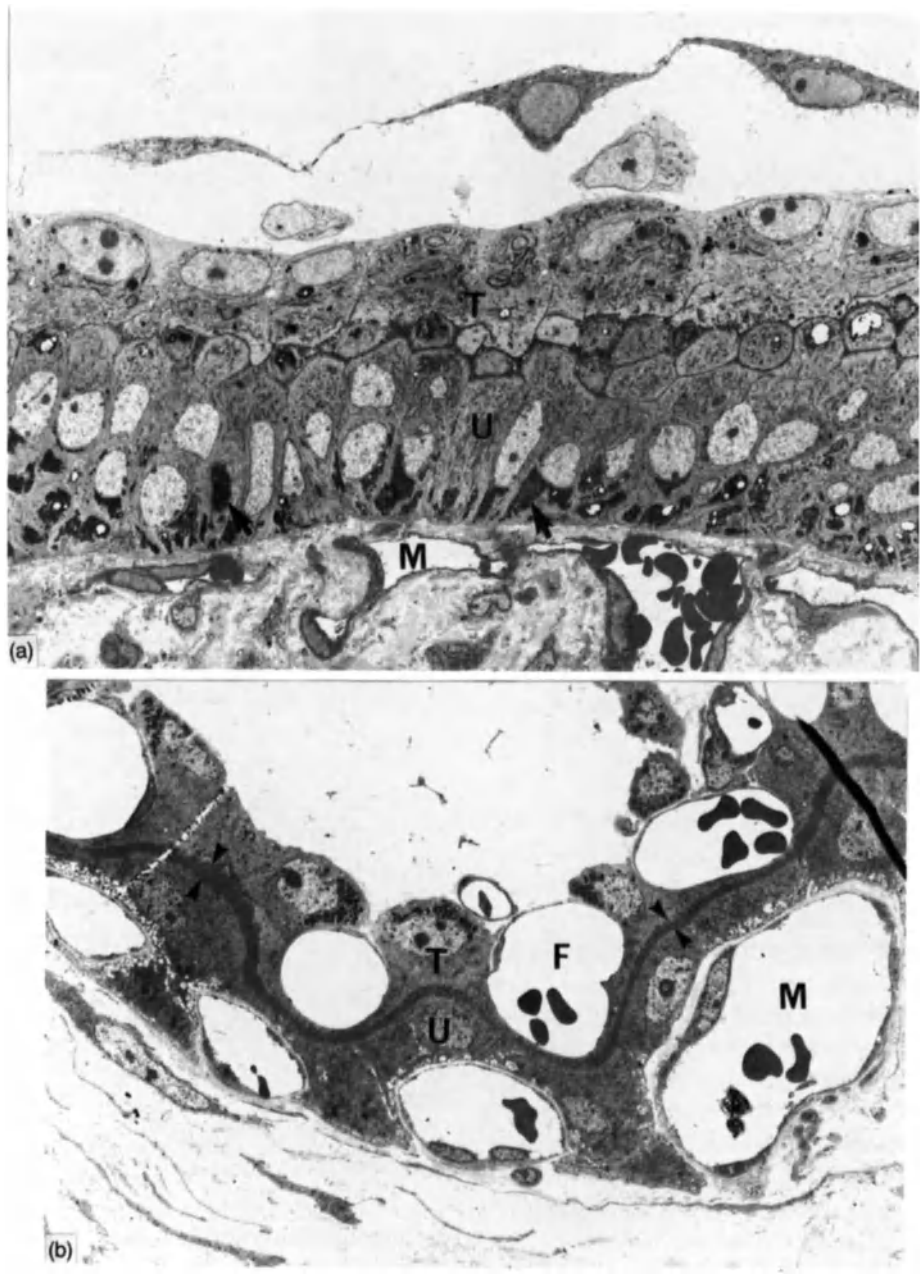


Figure 4.25 Epitheliochorial pig placenta. (a and b) Electron micrographs at the same magnification illustrating the development of the extreme indentation of the blood vessels into the trophectoderm (T) and uterine epithelial layers (U). The depth of the microvillar interdigitation can clearly be seen in (b) (arrowheads). F, Fetal vessels; M, maternal vessels; arrows, glycogen in uterine epithelium. (a) 16 dpc, × 400. (b) 109 dpc, × 400.

vascularizing trilaminar absorptive choriovitelline layer. By 25 dpc the allantois has formed a vascularized chorioallantoic cap over the embryo and its further expansion reduces the relative extent of the still absorptive choriovitelline layers. From 25 dpc a band of trophectodermal cells proliferates at the boundary between the chorioallantois and the trilaminar yolk sac to form the chorionic girdle (Figures 4.26 to 4.30).

By 40 dpc these cells have migrated into the maternal endometrium to form a ring of endometrial cups (Figures 4.26a and b and 4.28) and the yolk sac is reduced to a vestige within the umbilical cord. Subsequent growth of the conceptus is based entirely on the chorioallantois. Unlike the pig there is no amniochorion formation since the conceptus is isolated from the chorion by growth of the allantois (Figure 4.1). The umbilical cord is unusually long, having amniotic and allantoic portions linking the fetus to the membranes (Whitwell and Jeffcot, 1975; Ginther, 1979).

Development of the horse placenta The conceptus moves freely between the two horns of the uterus between 12 and 16 dpc as it expands from 12 mm to an 28-mm sphere. This has been demonstrated by ultrasonography and is presumably caused by contractions of the uterine musculature. Maternal recognition of pregnancy probably occurs between 14 and 16 dpc based on changes in prostaglandin content of the uterine vein (Sharp *et al.*, 1989). At 17 dpc the expanding conceptus reaches a critical diameter and fixes in the caudal portion of the horn which offers most luminal resistance (Ginther, 1983, 1984a,b; twin conceptuses, Ginther, 1989). It can subsequently be detected by rectal palpation as a uterine swelling. Recent evidence suggests that there is little or no subsequent migration because of closure of the uterus around the conceptus with development of a considerable muscular tone in the myometrium. Cellular interaction is initially limited by an acellular capsule around the embryo which is produced by the trophectoderm after the conceptus reaches the uterus (9 dpc) and persists until 21–22 dpc then ruptures and disappears (Betteridge, 1989). Subsequently, it is probably the bilaminar portion of the yolk sac which adheres to the uterine epithelium. The adhesion is tenuous and if the uterus is cut open between 22 and 40–50 dpc the conceptus is expelled by the internal pressure in the uterus (Ginther, 1979). There is a copious secretion from the uterine glands during this time (Zavy *et al.*, 1984), most of which is absorbed initially by the trilaminar yolk sac (from 16 to 28 dpc) and later by the chorioallantois.

From 27 to 35 dpc the avascular trophectoderm at the boundary between the chorioallantois and the trilaminar yolk sac proliferates to form a 'chorionic girdle' many cells thick (Figures 4.26a and b, 4.28, 4.29 and 4.30) (Allen, 1982; Enders and Liu, 1991a). Initial cell interaction (30–33 dpc) between mother and fetus is indicated by localized changes in lectin labelling of the uterine epithelium apposed to the developing girdle (Whyte and Allen, 1985). By 35 dpc the apical cells of the girdle have become binucleate, start to produce eCG (Figure 4.31b) and express MHC-1 antigens (Donaldson *et al*, 1990, 1992).

They then invade the uterine epithelium against which they are pressed by expansion of the conceptus against the firm uterine muscular tone (Figures 4.26, 4.30 and 4.31). The first binucleate girdle cells protrude pseudopia into and are said to push through the body of the uterine epithelial cells, well away from their tight junction seal (section 4.2.9c). They are then briefly delayed by the basement membrane of the uterine epithelium (along which they may migrate into uterine glands) and finally invade the endometrial stroma between the uterine glands (Allen *et al.*, 1973; Enders and Liu, 1991b) (Figures 4.26, 4.30 and 4.31). The uterine epithelium is subsequently phagocytosed

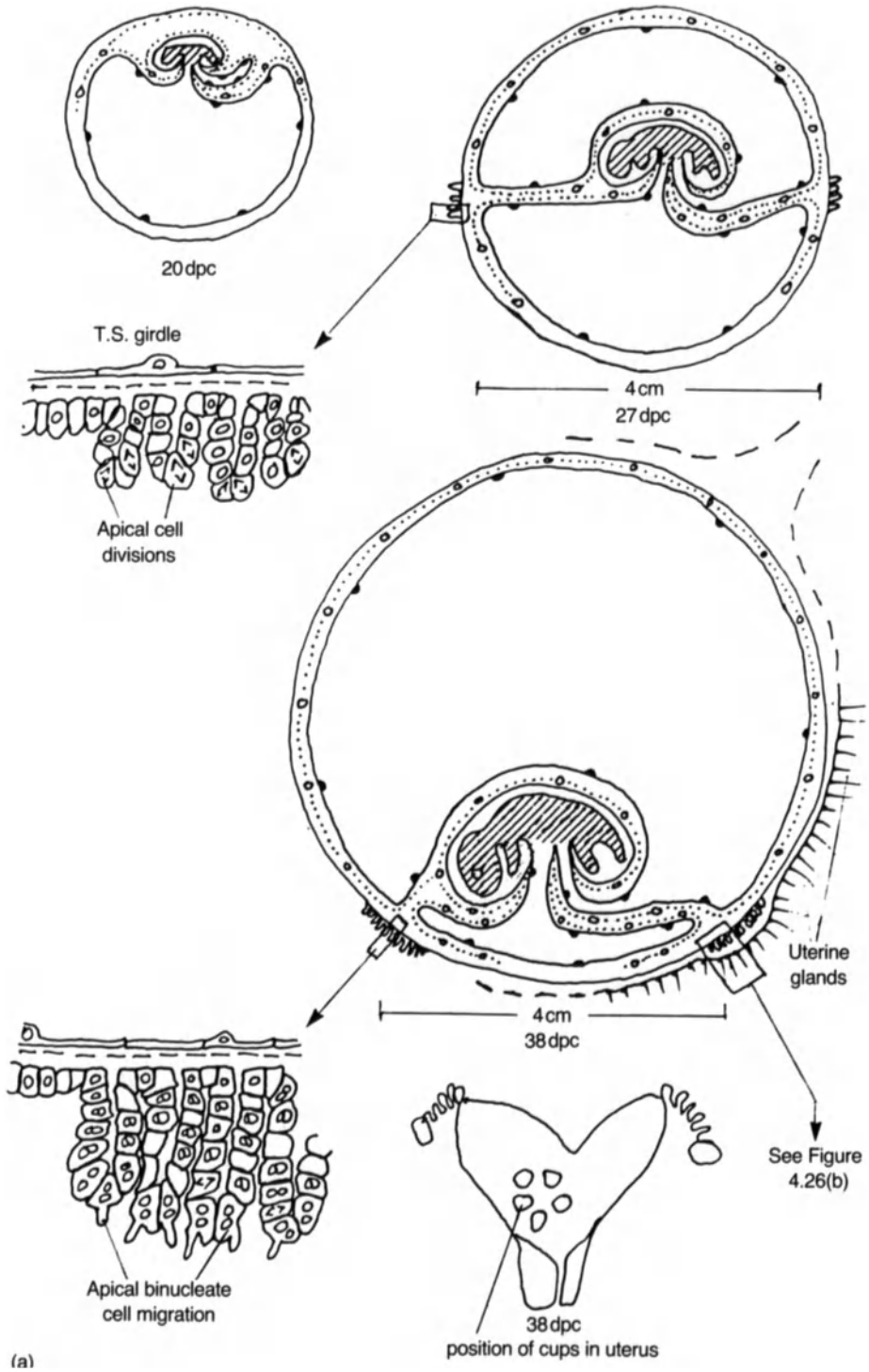


Figure 4.26 (a and b) The cellular changes in the development of the endometrial cups in Equidae.

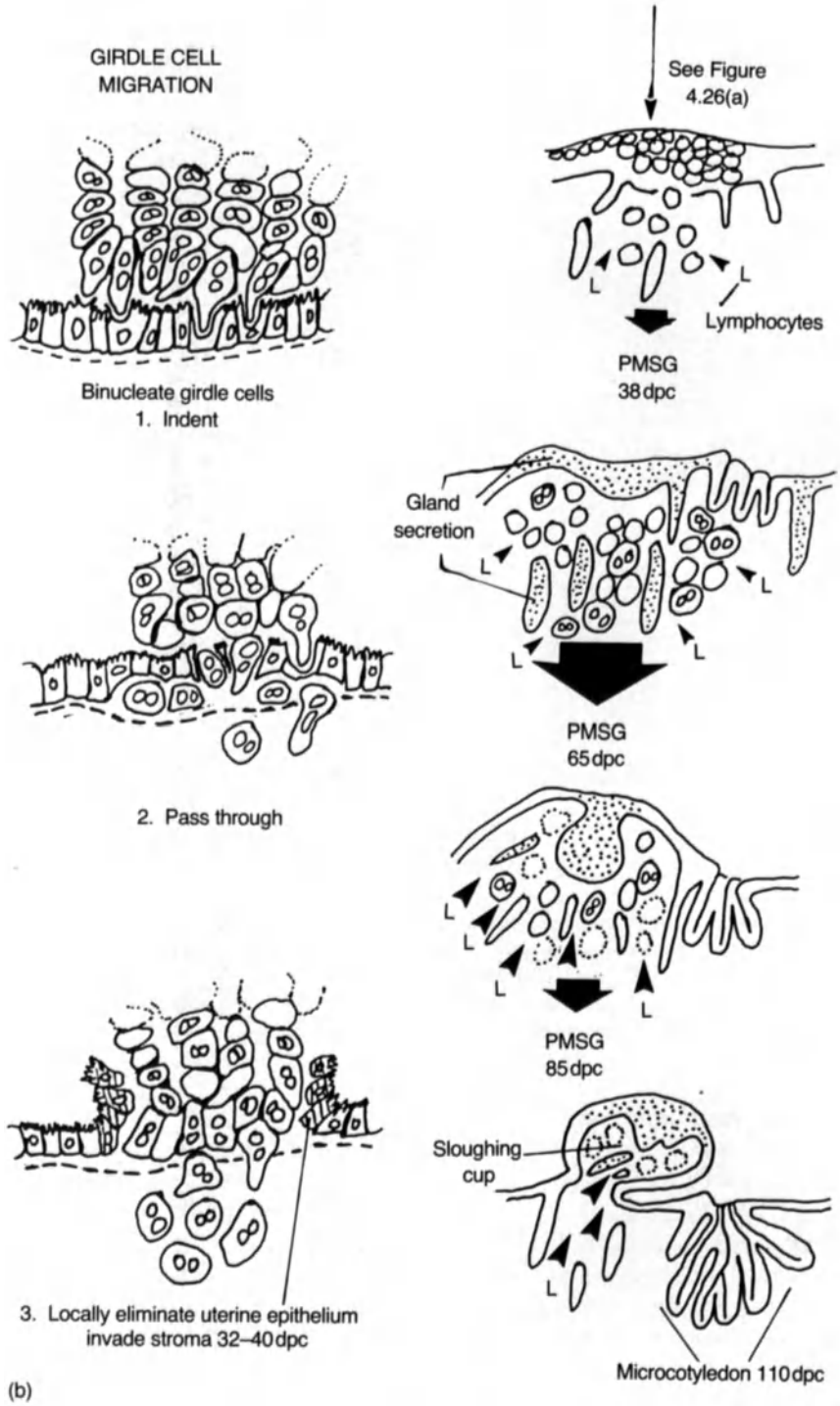


Figure 4.26 *continued*

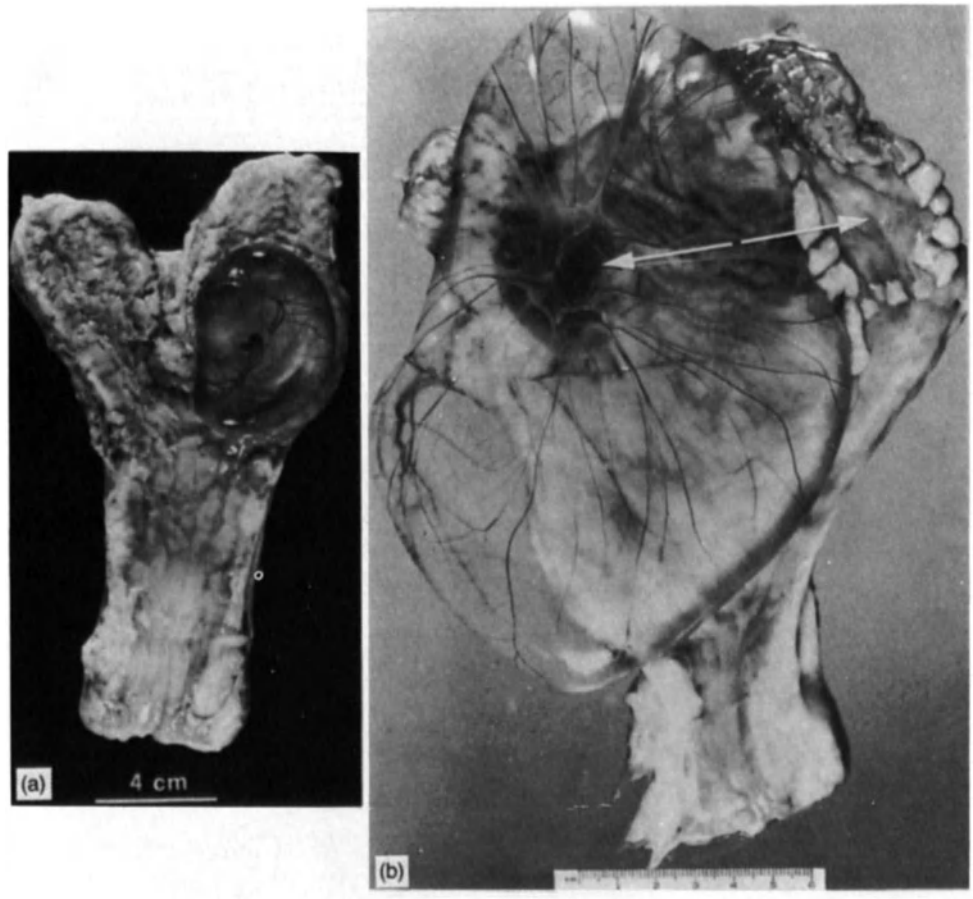


Figure 4.27 Horse conceptus, chorionic girdle and endometrial cups. The chorionic girdle forms between 28 and 36 days post coitum (dpc) around the conceptus in a band immediately above the junction of the allantois and yolk sac. The girdle remains the same size while the allantochorionic sac expands above it. Compare the diameter of the 30-dpc conceptus in (a) with the ring of cups in (b) from which the 60-dpc conceptus has been reflected (arrows). (a) 30 dpc, × 0.4. (b) 60 dpc, × 0.5. (From Ginther, 1979.)

and removed by continuing binucleate cell migration (Figure 4.31), which allows the whole chorionic girdle free access to the endometrial stroma (Figure 4.26).

Once in the uterine stroma (36–40 dpc) the binucleate cells have not been seen to divide but develop into masses of very large cells forming a ring of 'endometrial cups' (Figures 4.26 and 4.32).

The uterine epithelium grows in from the sides to cover the cup area. These cup cells secrete eCG which appears in the maternal (but never the fetal) circulation via the capillaries and lymphatic vessels in and around the cup. The mature cup cell has a cytoplasm full of mitochondria, rough endoplasmic reticulum cisternae and a large Golgi apparatus (D.W. Hamilton *et al.*, 1973) (Figures 4.33 and 4.34).

Ultrastructural immunogold studies have shown that eCG is localized in the Golgi cisternae and in small dense granules similar to those found in the migrating girdle cell and present both in the Golgi region and at the

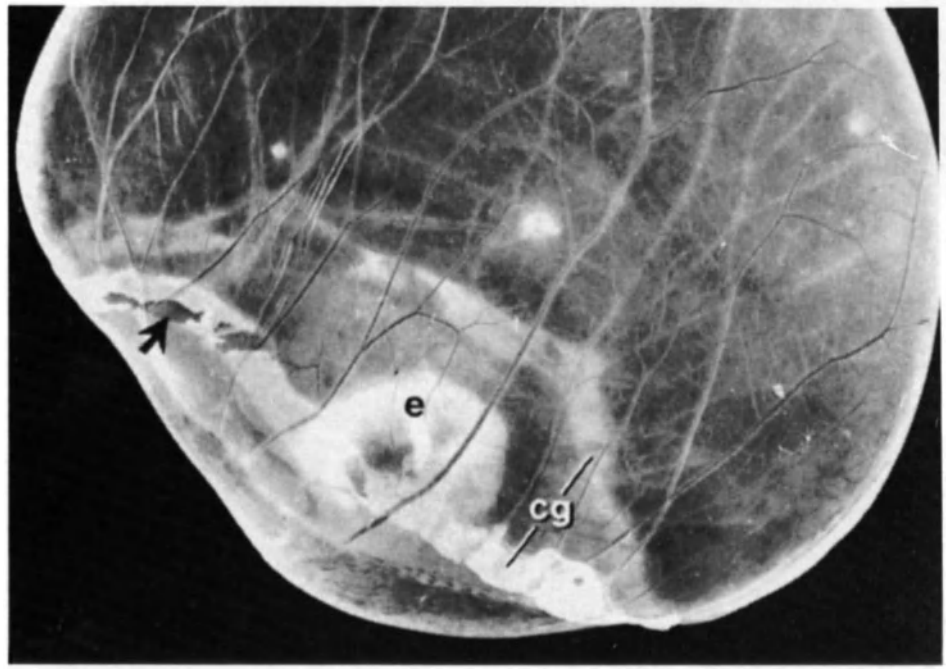


Figure 4.28 Horse conceptus, chorionic girdle. A 37-dpc conceptus with a clearly visible girdle (cg) from which some girdle cells have been lost on removal from the uterus (arrow). e, embryo. 37 dpc, × 2. (From Ginther, 1979.)

peripheral plasmalemma (Figures 4.31 and 4.34). Release of eCG would therefore seem to be by the usual exocytotic mechanism as found for other protein hormones. The amount of eCG produced depends on the size of the cup, which is determined by the number of fetal binucleate cell migrants. This last seems to depend upon the genotype of the conceptus. For example horse ♀x horse and horse ♀x donkey crosses produce large cups, while donkey ♀x donkey or donkey ♀x horse produce small cups (Allen, 1982).

Cells scraped from the girdle between 36 and 37 dpc will grow in tissue culture to form large, usually binucleate, cells which produce considerable amounts of eCG for up to 200 days (Allen and Moor, 1972; Figure 4.33b). On the analogy of the ruminant binucleate cell it seems unlikely that binucleate girdle cells can divide, so any increase in cell numbers in culture would have to be based on division of the uninucleate (stem?) cells of the girdle. Fetal cells, already binucleate, isolated from endometrial cups will not divide or persist successfully in culture.

Maternal lymphocytes and macrophages start to accumulate at the edge of a cup as soon as it establishes itself (Figure 4.32). Maximum secretion of eCG from the cup occurs between 50 and 80 dpc, after which the lymphocytes start to invade and kill the cup cells. The uterine glands in the cup also produce a considerable amount of secretion at this time (Figure 4.32), presumably stimulated by the cup cells. This glandular secretion contains very large amounts of eCG secreted by the cup cells, but surprisingly this does not reach the fetal circulation.

The variation of cup size with genotype, the lymphocyte accumulation and the ability of the cup cells to secrete eCG in tissue culture (Figure 4.33) for up to 200 days suggests

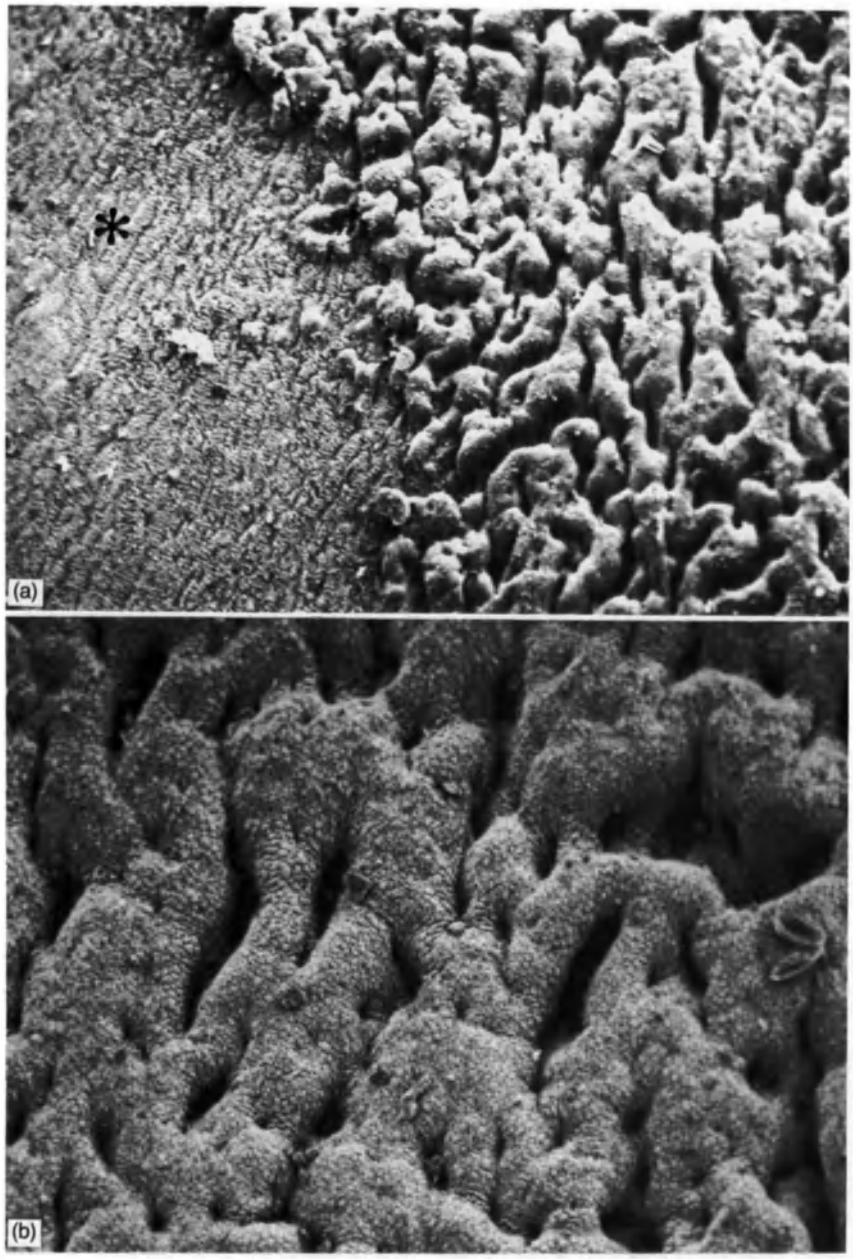


Figure 4.29 Horse conceptus, chorionic girdle. (a) Scanning electronmicrograph of a 32 dpc girdle. Note the very sudden transition from smooth trophectoderm (asterisk) to the convolutions of the girdle. (b) Detail from (a) showing how the girdle cell proliferation forms microfolds and crevices. (a) 32 dpc, × 60. (b) 32 dpc, × 170. (Courtesy of Dr D.F. Antczak.)

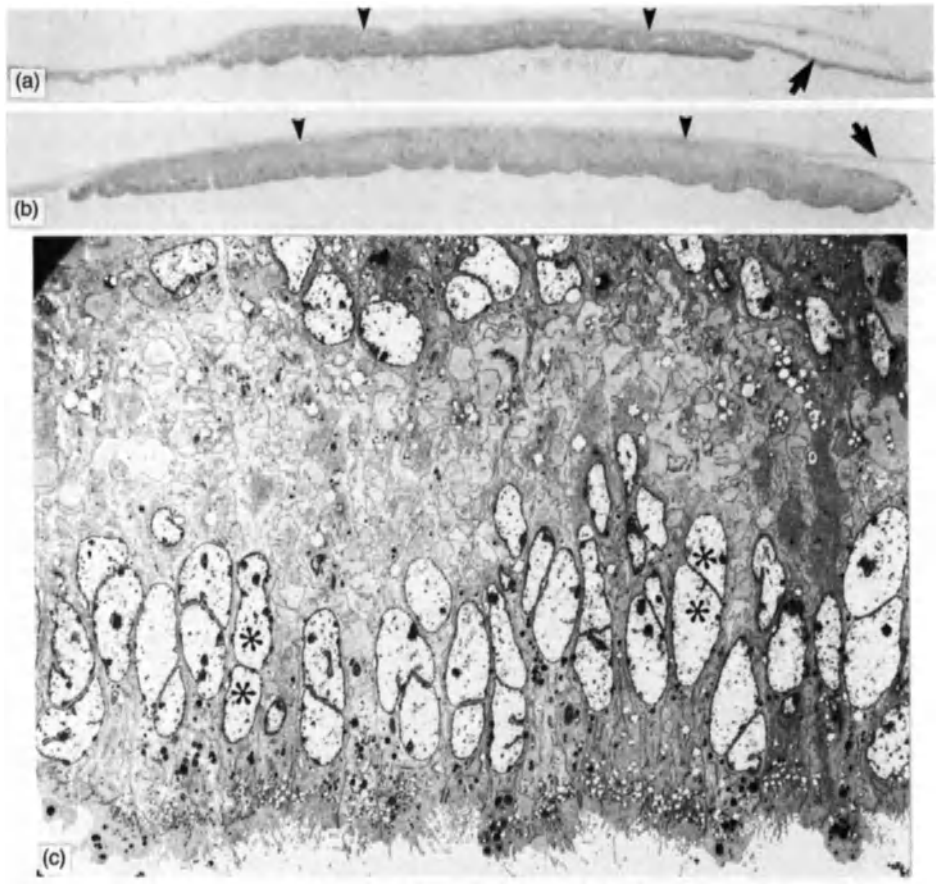


Figure 4.30 Horse conceptus, chorionic girdle. Cross sections through (a) 32 and (b) 36 dpc girdles showing the considerable proliferation of the trophectodermal cells but the basement membrane (arrowheads) remains flat. Compare the thickness of the unmodified trophectoderm outside the girdle (arrows): (c) Electron micrograph of the apex of fetal chorionic girdle at 37-dpc. Most of the cells are binucleate (e.g. asterisks). (a) 30 dpc, × 25. (b) 36 dpc, × 25. (c) 37 dpc, × 1100. (a and b) courtesy of Dr W.R. Allen.

that the cytolysis and regression of the cup cells is caused by an immunological reaction rather than a programmed cell death (Allen, 1982). Subsequently, the whole mass of degenerating cup tissue is everted from the endometrium, encapsulated by the overlying allantochorion, and remains as a necrotic residue in the allantoic cavity (Whitwell and Jeffcot, 1975). The uterine epithelium grows over the scar from the edges (Ginther, 1979).

Interspecific embryo transfer experiments (e.g. zebra conceptus into horse, Summers *et al.*, 1987) have shown that as long as there is some cup development, however small, normal gestation results and usually 'pregnancy-specific antibody' is elicited in the maternal blood. However, a donkey conceptus transferred to a horse produces neither a cup reaction nor pregnancy antibody and abortion usually follows between 80 and 100 dpc in the presence of a massive lymphocyte infiltration throughout the entire endometrium, ostensibly similar to the infiltration round a normal size (horse in horse) cup. In some

cases this abortion can be blocked by infusing pregnant mare's serum (but not eCG alone) or paternal lymphocytes into the mare between 20 and 80 dpc (Allen *et al.*, 1987).

Once a horse has carried one donkey pregnancy successfully (and this is as yet entirely unpredictable) with or without treatment, then most subsequent donkey conceptuses are carried to term without further therapy – the system displays immunological memory.

The girdle and the early cup cells are the only allantochorionic cells to express MHC antigens (Crump *et al.*, 1987; Donaldson *et al*, 1990, 1992). Presumably, it is this exposure to MHC (and other trophoblast antigens) which stimulates the maternal immune system to produce the antibodies and/or recruit suppressor lymphocytes necessary for a successful pregnancy.

At 35–38 dpc the allantois has vascularized

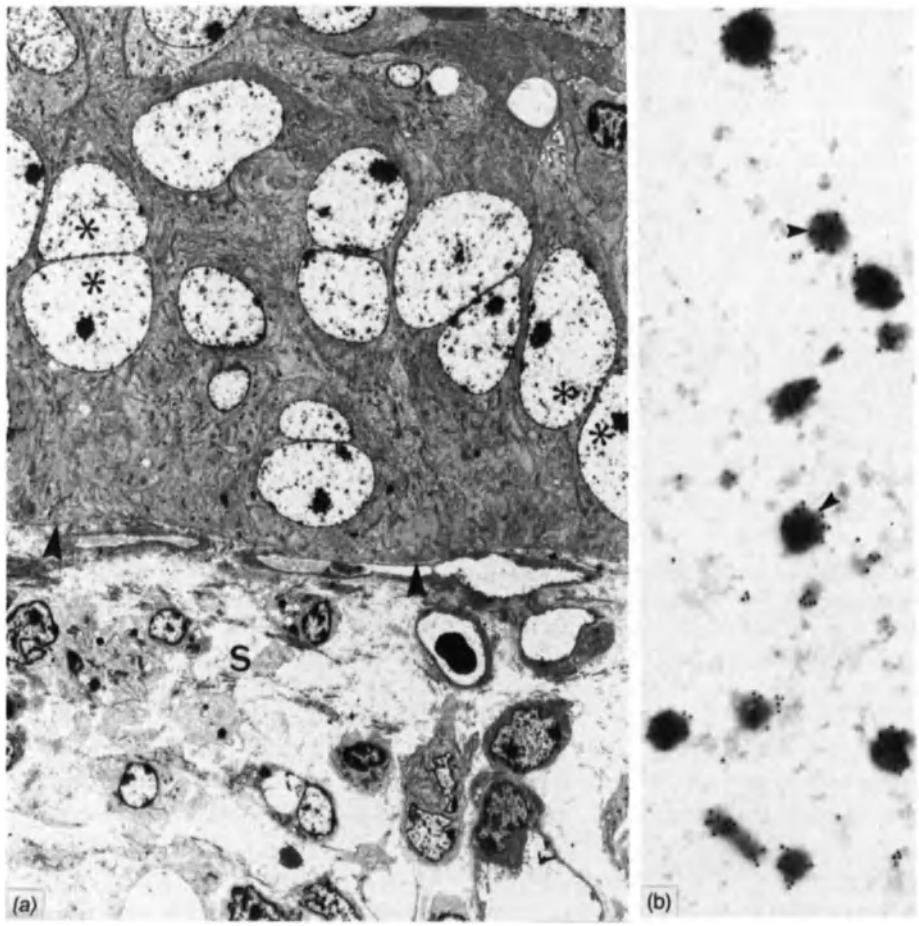


Figure 4.31 Horse chorionic girdle. (a) The fetal chorionic girdle has invaded and eliminated the uterine epithelium but further migration into the maternal endometrial stroma (S) is delayed by the residual basement membrane (arrowheads) of the epithelium. Most girdle cells are binucleate (asterisks). 37 dpc, × 1700. (b) Binucleate girdle cells in the position shown in (a) often contain groups of small granules. This section has been processed with immunocytochemical methods to demonstrate that the granules contain equine chorionic gonadotrophin indicated by the gold colloid marker (arrowheads). 37 dpc, × 30 000.

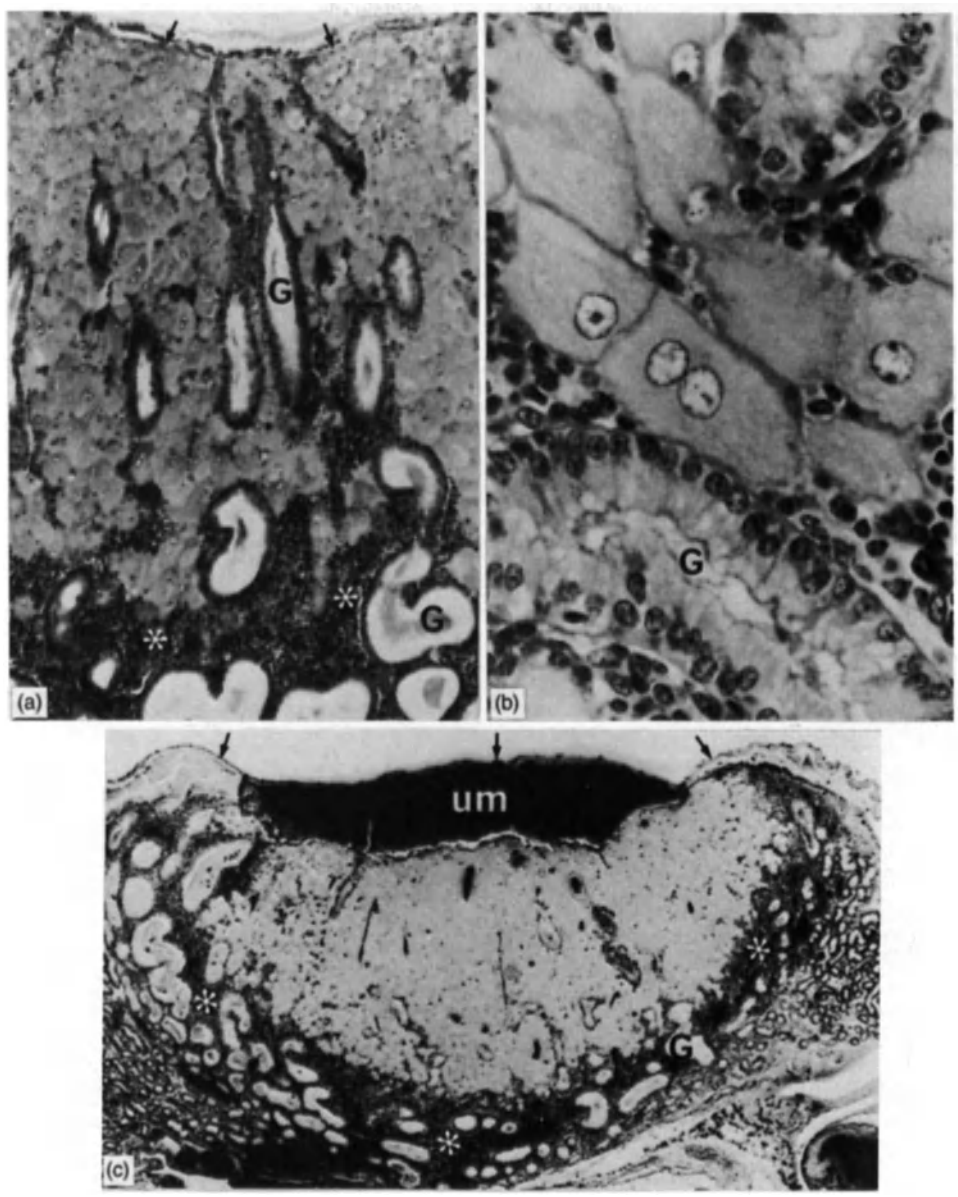


Figure 4.32 Horse endometrial cup structure. (a) Section through a newly formed cup at 40 days post conception (dpc) with cup cells closely packed around the uterine glands (G). The uterine epithelium (arrows) reforms rapidly after migration of the girdle cells is completed at 38–40 dpc. There is an accumulation of lymphocytes around the base of the cup (asterisks). (b) Note the large size of the cup cells compared with the gland cells (G). (c) Mature cup (60 dpc) with accumulated secretion (um) localized between the allantochorion (arrows) and the cup. Considerable numbers of lymphocytes have accumulated in a continuous zone (asterisks) around the cup. (a) 40 dpc, × 70. (b) 40 dpc, × 2000. (c) 60 dpc, × 30. (a–c from Ginther, 1979.)

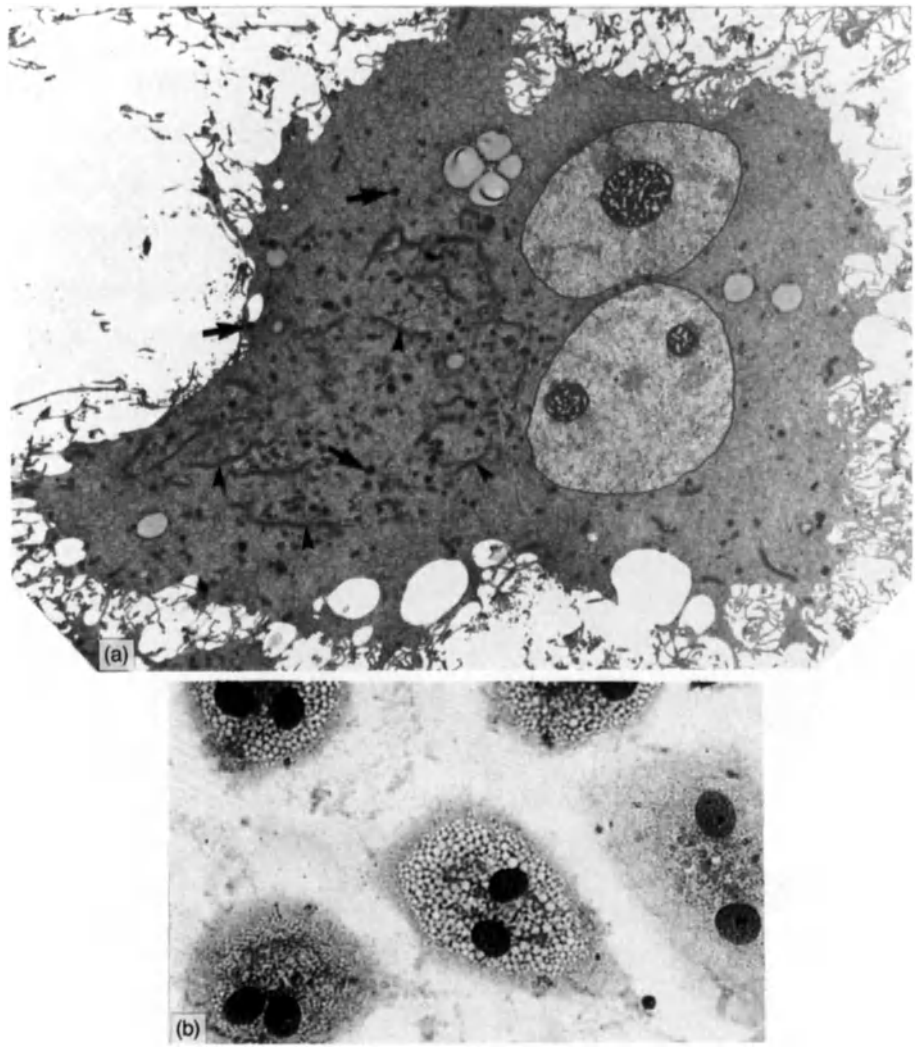


Figure 4.33 Horse endometrial cup cells (a) *in vivo* and (b) *in vitro*. (a) An electron micrograph of a characteristic binucleate cell from a 50-dpc cup fixed by perfusion *in situ*. The material was processed without osmium and stained with phosphotungstic acid (Wooding, 1980). This emphasizes the two nuclei and extensive Golgi cisternae (arrowheads) and small granules (arrows) in a typical endometrial cup cell. 50 dpc, × 3200. (b) Light micrograph of cells isolated from a 35-dpc girdle and grown in tissue culture for 50 days. They are very large, characteristically binucleate and produce considerable amounts of eCG. The cytoplasm accumulates numerous lipid droplets giving a stippled appearance, × 1300. (Courtesy of Dr R. Moor.)

more than 90% of the total trophectoderm, and this is the earliest stage at which microvillar interdigitation between uterine epithelium and trophectoderm has been reported (Steven, 1982). The fetomaternal cell sheets then produce villi by a process of complementary growth. Fetal villi are formed over the entire surface of the conceptus except over the openings of the numerous endometrial glands. Here the trophectoderm

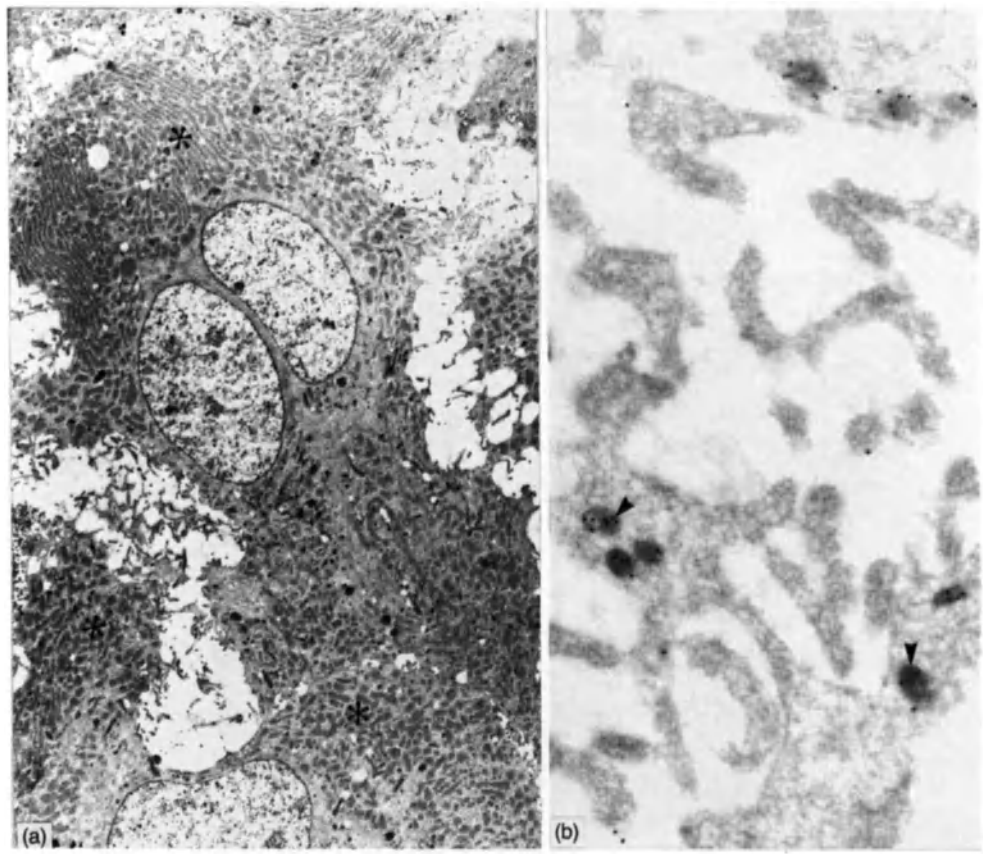


Figure 4.34 Horse endometrial cup cells. (a) Electron micrograph of cells processed without osmium and stained with phosphotungstic acid followed by lead citrate which enhances the massive arrays (asterisks) of rough endoplasmic reticulum cisternae filling most of the cytoplasm of the cup cells. 50 dpc, × 3200. (b) The small granules produced by the Golgi apparatus of the cup cells (see Figure 4.33a) contain eCG as indicated by the immunogold colloid marker (arrowheads). They are usually located peripherally but never seem to accumulate in any number. 50 dpc, × 44 000.

develops a dome of highly absorptive columnar cells over the gland mouth and its secretion, forming areolae exactly as in the pig, if a little smaller in size (Figure 4.35).

By 60 dpc the fetal villi are longer and have started to group together between the areolae. Further extension of the villi with secondary branching plus the development of a bounding maternal connective tissue capsule produces the characteristic microcotyledon between the glands, each 1–2 mm in diameter at term (Samuel *et al.*, 1974) (Figures 4.26, 4.35 and 4.36).

After girdle cell migration has finished (about 40 dpc) there is no published evidence for any specialized cells or any syncytium in the trophectoderm or uterine epithelium. Like the pig, the trophectoderm is uniformly uninucleate and its microvilli are closely interdigitated with the uniformly uninucleate uterine epithelium. This fetomaternal junctional area increases (as does fetal weight) throughout pregnancy by mutual growth of the fetal and maternal epithelia (Baur, 1977), and both are increasingly indented by their initially subepithelial capillaries (Samuel *et*

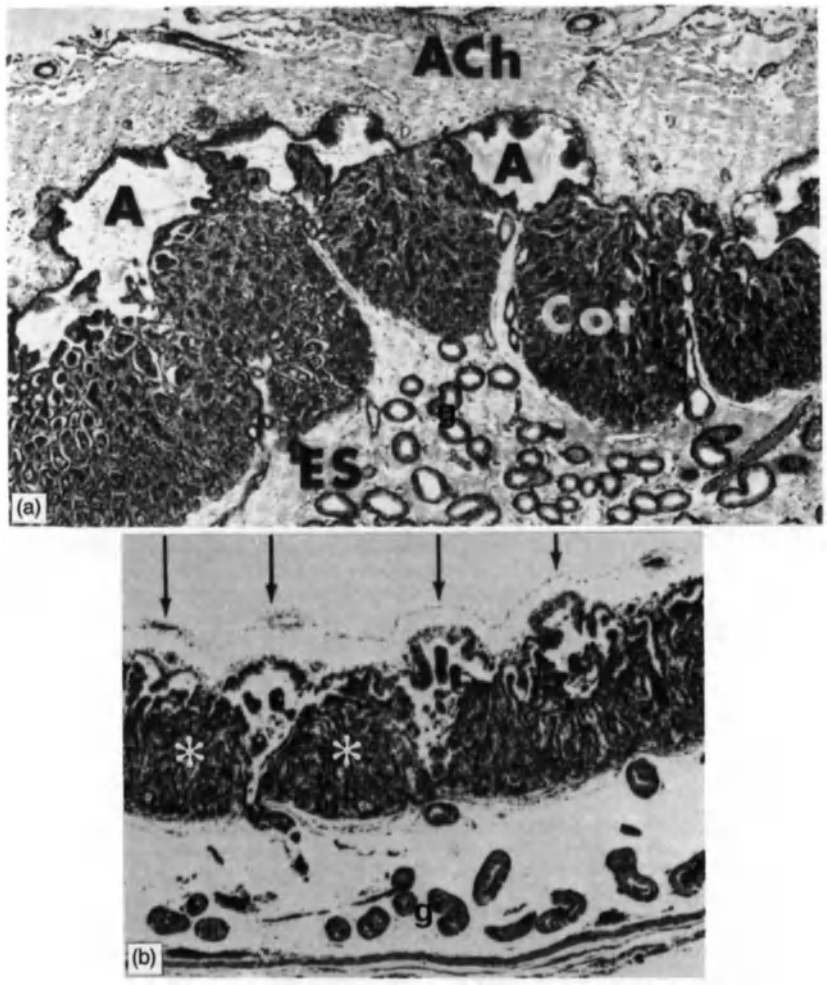


Figure 4.35 Epitheliochorial placentas. The horse (a) with its microcotyledons (Cot) with areolae (A) between in the allantochorion (ACH) has a very similar placental architecture to the American mole, *Scalopus* (b). In (b) the areolae are indicated by arrows, the microcotyledons by asterisks. In the endometrium (ES) of both there are many glands (g) which empty into the areolae. (a) Mid-pregnancy, × 20. (From Bjorkman, 1970.) (b) Late gestation, × 37. (From Prasad *et al.*, 1979.)

al., 1976). Indentation is most marked in the fetal trophectoderm but is not so extreme as observed for the pig.

There are no extravasations of maternal blood at the microvillar junction (as found in ruminants and carnivores) so iron transport is presumably based on a similar carrier system to the pig protein uteroferrin in the glandular secretions. A similar protein has been reported in the horse (Zavy *et al.*, 1979, 1984).

The trophectodermal cell fine structure changes during pregnancy. At 200 dpc there is a considerable amount of smooth endoplasmic reticulum and this persists to term. Rough endoplasmic reticulum is found from 180 dpc to term, when the number of dense bodies (probably lysosomal) has also increased considerably (Samuel *et al.*, 1976).

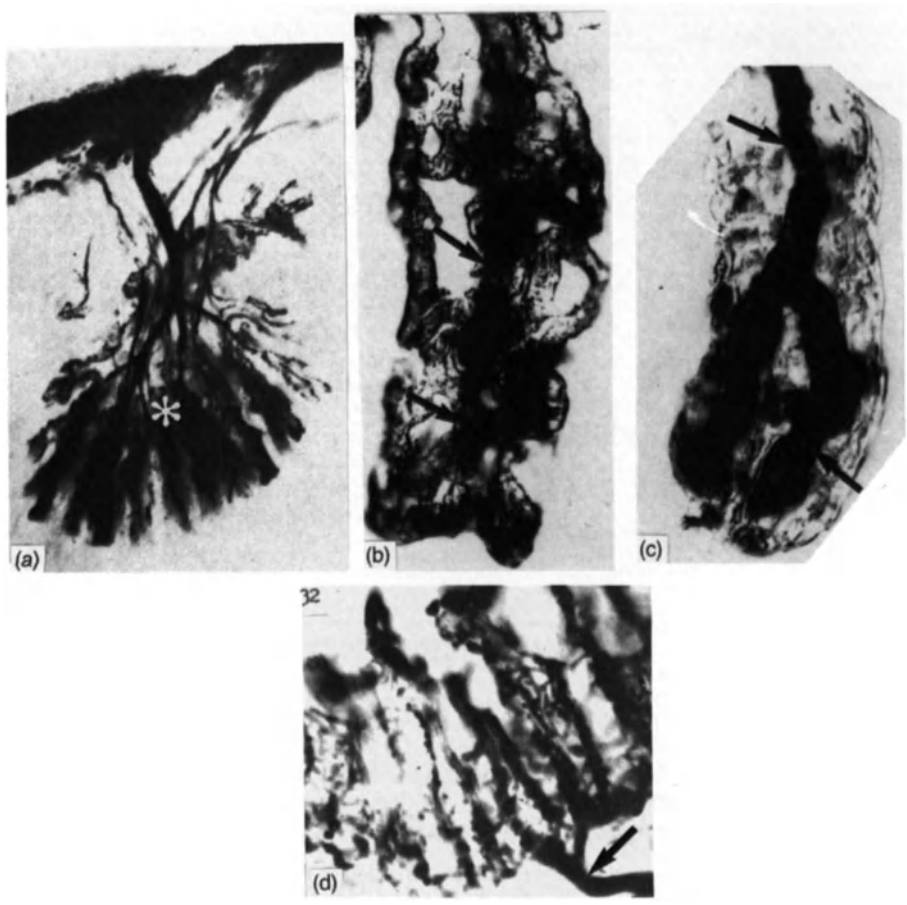


Figure 4.36 Horse epitheliochorial placenta. (a–d) Vascular injections illustrate the tuft of fetal vessels forming a microcotyledon (asterisk), and partly filled villus tips show that there is a central arteriolar supply (arrows, b and c). (d) The capillary network forming the maternal cotyledonary blood supply with a large draining venule (arrow). (a) 210 dpc, × 40. (b) 195 dpc, × 400. (c) 195 dpc, × 400. (d) 150 dpc, × 70. (All from Tsutsumi, 1962.)

The endocrine profile of the horse during pregnancy is complex (Allen, 1975, 1982) (see Figure 4.37).

A peak level of 10 ng/ml serum progesterone is initially produced at 8 dpc by the primary corpus luteum, but this secretion gradually decreases. The eCG produced by the endometrial cup cells starting at 38 dpc then stimulates ovulation and the production of the secondary corpora lutea, which increase the progesterone levels considerably (up to 14 ng/ml). As the cups and secondary corpora lutea regress (100–150 dpc) the placenta takes over production of a much reduced level of progesterone (3–4 ng/ml), presumably related to the considerable amount of smooth endoplasmic reticulum in the trophectoderm of the microcotyledons. Oestrogens are also produced, mainly oestrone and the unique equid B-ring unsaturated oestrogens equilin and equilenin (Allen, 1975; Pashen *et al.*, 1983; Haluska and

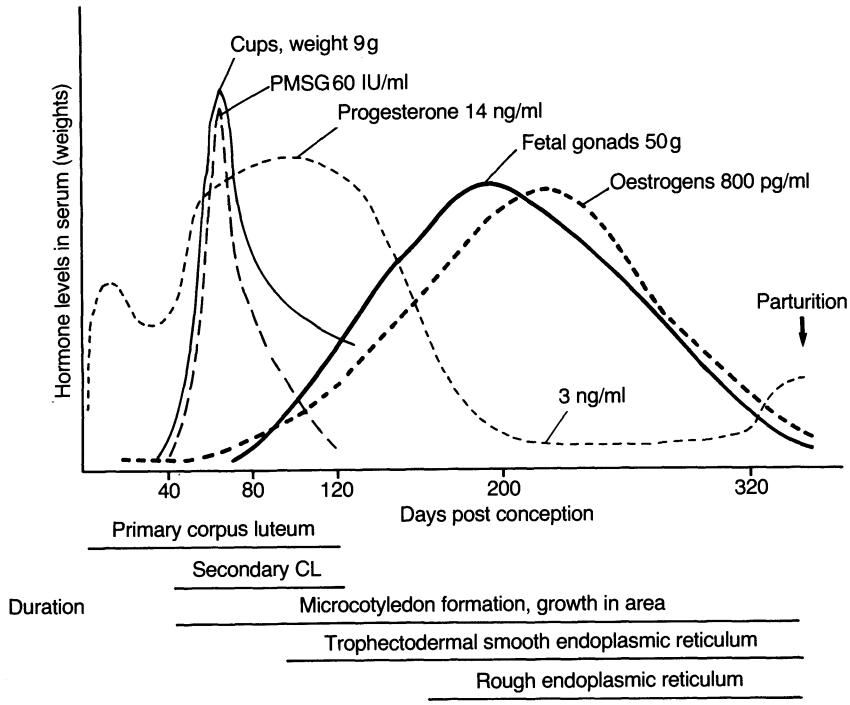


Figure 4.37 Comparison of the endocrine changes in the serum and the development of endometrial cups and fetal gonads during pregnancy in the horse. Note that the lifespan of the corpora lutea correlates with high progesterone levels; the levels necessary to support pregnancy in second half of gestation are much lower and are supplied by placental synthesis. (Data from Ginther, 1979, and Samuel *et al.*, 1974, 1976.)

Currie, 1988). Precursors for the oestrogens are produced by the hypertrophied interstitial cells in the **fetal** gonads (Figure 4.37).

The pattern of blood vessels is very different in the horse fetal villus when compared with that in the pig. There is a central arteriole which passes directly to the villus tip before dividing into a peripheral capillary network draining back down the villus to its base (Figures 4.9 and 4.36). The maternal arteries run first to the tips of the maternal villi or septa which are inserted between the bases of the fetal villi. The blood then drains down through a meshwork of peripheral capillaries to the collecting veins at the base of the maternal villus (septum) (Tsutsumi, 1962; Steven, 1982) (Figures 4.9 and 4.36).

This arrangement should allow classical countercurrent flow between fetal and maternal capillaries assuming they run alongside one another for a sufficient distance. At parturition the interdigitated microvilli of the fetal and maternal cells separate by a process probably requiring changes in adhesion at the molecular level. The short fetal villi and maternal septa (villi) then disengage, probably as a result of decrease in blood pressure and/or volume. There is rarely any problem with separation and expulsion of the membranes at partus (Steven *et al.*, 1979). Details of the decrease in systemic progesterone (Bazer and First, 1983) and increases in prostaglandin and oxytocin at this time are given in Haluska and Currie (1988).

Other epitheliochorial types *Scalopus aquaticus*, the American mole, is an insectivore with a bicornuate uterus, producing an average litter of four after 35 days of gestation. Implantation is superficial with no apparent decidual formation, the amnion forms by folding and there is a functional yolk sac until term. The definitive chorioallantoic placenta is diffuse villous and epitheliochorial with numerous areolae (Prasad *et al.*, 1979).

Scalopus is the only insectivore known with an epitheliochorial placenta, most are haemochorial with a few endotheliochorial examples (Mossman, 1987). However, they all have a similar yolk sac development, with a mesometrial bilaminar yolk sac which persists to term and a functional trilaminar choriovitelline placenta on the lateral and antimesometrial, glandular uterine walls. The vitelline capillaries are displaced by development of the allantoic circulation to produce the simple villi. The chorioallantoic villi are collected into groups similar to the microcotyledons of the equid type but they lack the connective tissue capsule (Figure 4.35b). The uterine epithelium is extremely thin (< 1 μm) in places and required EM studies to prove that it persists (Prasad *et al.*, 1979). Areolae develop over the mouths of the uterine glands separating the villus groups. At parturition, separation occurs at the fetomaternal microvillar junction.

Scalopus with its epitheliochorial placenta has obvious skeletal and developmental affinities with insectivores such as *Talpa*, the European mole (endotheliochorial placenta), and with the shrews and the European hedgehog (haemochorial placentas). This emphasizes the point that animals of very similar phyletic origins, size, habitat and reproductive behaviour can have widely differing definitive placental structures. Alternatively, it may be taken as a demonstration that the transition from epitheliochorial through endotheliochorial to haemochorial is a relatively simple developmental change.

Another very different order, Pholidota (scaly anteater or pangolin) has a similar epitheliochorial placenta to Equidae with grouped short villi between frequent areolae (Mossman, 1987).

Cetacea, the whales, porpoises and dolphins, have diffuse epitheliochorial placentas like that of the pig with short villi or folds and numerous areolae opposite the mouths of uterine glands. None of these have yet been investigated in detail with the EM (Mossman, 1987).

The strepsirhine primates, lemurs, lorises and bushbabies (galago), all have diffuse epitheliochorial placentation based on narrow slender or short fat individual villi with large bulbous areolae between (Figure 4.20d and e; compare with Figure 4.21, pig). At implantation *Galago senegalensis* is reported to produce a specialized area of giant trophoblast cells which remove the uterine epithelium and act as an anchorage point for the conceptus (Butler, 1967). This is reminiscent of the trophectodermal protrusions into the uterine epithelium produced by some of the marsupials (Enders and Enders, 1969). In both cases these giant cells are transient and do not contribute to the definitive placental development. Whether the implantation is produced by delamination intrusion or fusion is not yet clear. In the definitive placenta of the lemur *Microcebus* a discoid structure with no uterine epithelium (Grosser's syndesmochorial) has been claimed (Reng, 1977), but insufficient detail can be seen to verify this; and a giant, possibly double, cell layer has been suggested for *Galago demidovii* (Mossman, 1987). Both these structures would be very atypical and it needs EM resolution to unravel the details. A recent ultrastructural study of the definitive placenta of *Galago crassicaudata* emphasized the simplicity of the basic epitheliochorial villus pattern (B.F. King, 1984).

The Camelidae and Tragulidae (chevrotains) are reported to have large branched folds and villi forming diffuse epitheliochorial placentas (Morton, 1961). What is more

important is they are said to have some (Camelidae) (Lennep, 1961) or many (Tragulidae) (Mossman, 1987) binucleate cells in the trophectoderm. It has been suggested that these BNCs may be equivalent to ruminant BNCs, capable of migration and fusion to form fetomaternal hybrid tissue at the fetomaternal interface (section 4.2.6b). However, a recent EM study on the camelid, llama, could find no evidence for any significant numbers of BNCs with the characteristic ruminant structure (Steven *et al.*, 1980a). Any uninucleate epithelium or tissue normally produces a low level (1–3%) of cells with two nuclei, but these have identical ultrastructure to their uninucleate neighbours. The Camelidae BNCs are probably in this category (Lennep, 1961). The Tragulidae report needs further investigation, as it suggests that there are as many BNCs (15–20%) as are normally found in a ruminant but present in a diffuse noncotyledonary placenta. This might provide valuable clues as to why placentomes form.

(b) Synepitheliochorial placentation (sheep, cow)

In Grosser's syndesmochorial category (almost exclusively ruminants) the uterine epithelium was said to be lost and the trophectoderm apposed to the maternal connective tissue directly. Further work demonstrated that the uterine epithelium persisted to a variable degree but was considerably altered. It has recently been shown by a variety of techniques that ruminant fetal trophectodermal BNCs invade the uterine epithelium and fuse to form fetomaternal hybrid syncytial plaques at the junction of the fetal and maternal tissue (Wooding, 1982a,b, 1984) (Figures 4.38 and 4.39).

This is so different from the epitheliochorial category that it amply justifies a separate grouping.

'Syndesmochorial' is of obscure derivation and we propose synepitheliochorial as a much more descriptive and easily understood category (section 4.2.3c). All ruminant placentas so far investigated have BNCs which produce the fetomaternal hybrid syncytium with uterine epithelial cells but the degree to which the maternal epithelium is significantly altered does vary. The 'syn-' indicates the contribution of the BNC-derived syncytium and the retention of 'epitheliochorial' emphasizes that large areas of simple fetomaternal apposition are present in the definitive placenta (Wooding, 1992).

The synepitheliochorial placenta has three identifying characteristics: the BNC in the fetal trophectoderm (section 4.2.7b) which form a structurally separate population among the uninucleate trophectodermal cells from which they are derived; the fetomaternal syncytium formed by BNC fetal migration and fusion with some or all of the maternal uterine epithelial cells; and the cotyledonary chorioallantoic placental organization, with the restriction of fetal and maternal villus elaboration to discrete regions (placentomes) based on pre-existing non-glandular caruncular areas on the uterine epithelium (Amoroso, 1952) which are well developed in the fetal lamb uterus by mid-pregnancy (Wiley *et al.*, 1987). A placentome is formed by enmeshed fetal villi and maternal crypts; the tuft of fetal villi is correctly referred to as a cotyledon and the corresponding maternal structure is a caruncle (Figures 4.40 and 4.41).

However, cotyledon is also frequently used for the whole structure, so that a 'maternal cotyledon' corresponds to a caruncle. Ruminant placentas may be oligocotyledonary with 3–8 large placentomes, as in deer, or polycotyledonary with numerous (20–150) but smaller placentomes as in cows, goats and sheep (Figure 4.4). Since there are a finite number of caruncles per uterus, although a singleton conceptus may not utilize all the sites it will have more placentomes than twins, which can only have about half the total number each. However, the twins will have larger individual placentomes and their

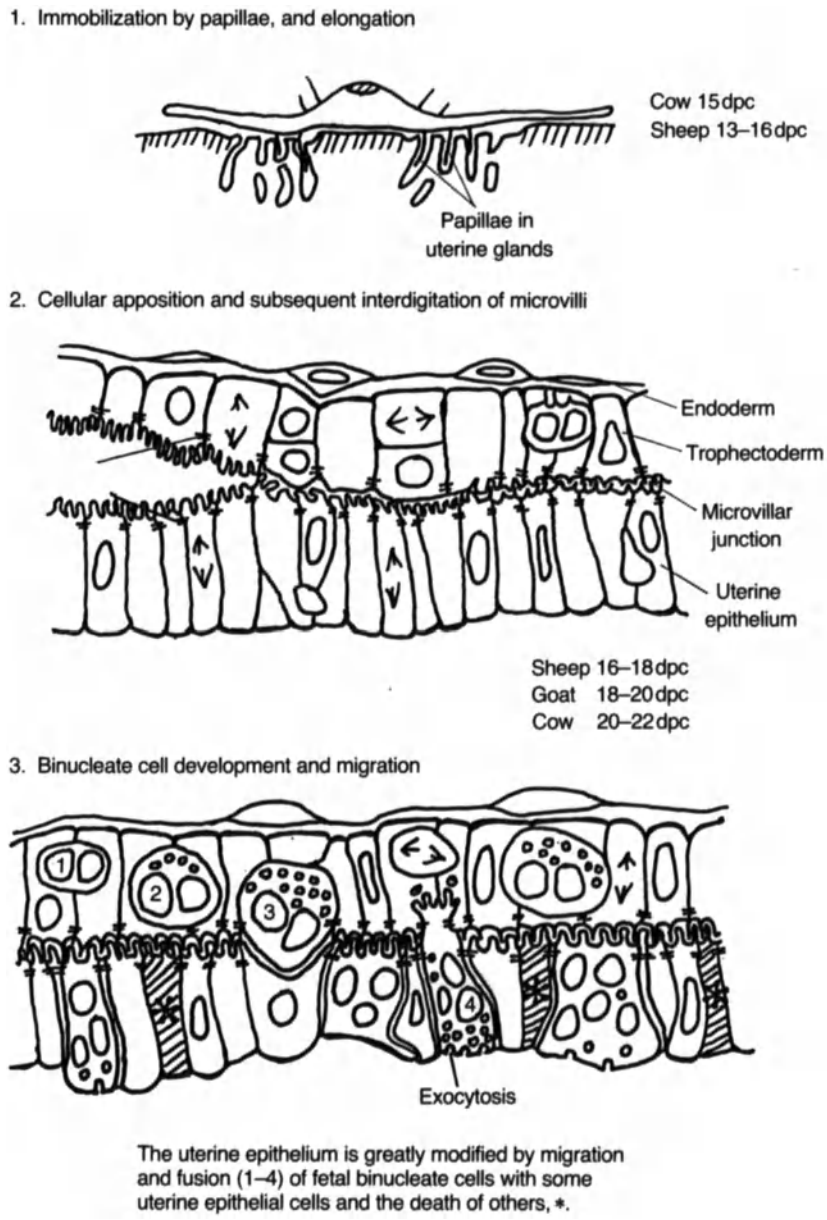


Figure 4.38 The cellular changes at implantation in the ruminants.

combined mass will be greater than that of the singleton. There is a close correlation between conceptus placentomal mass and birthweight of the fetus (A.W. Bell, 1984, 1991), and recent studies indicate a pivotal role for IGF-1 and -2 in placental growth (Bassett, 1991; Geisert *et al.*, 1991; Owens, 1991). Placentomes also differ in gross structure, being concave to the fetal side in sheep and goats, but convex in the cow and flat in antelopes (Amoroso, 1952). The differences are probably without functional significance

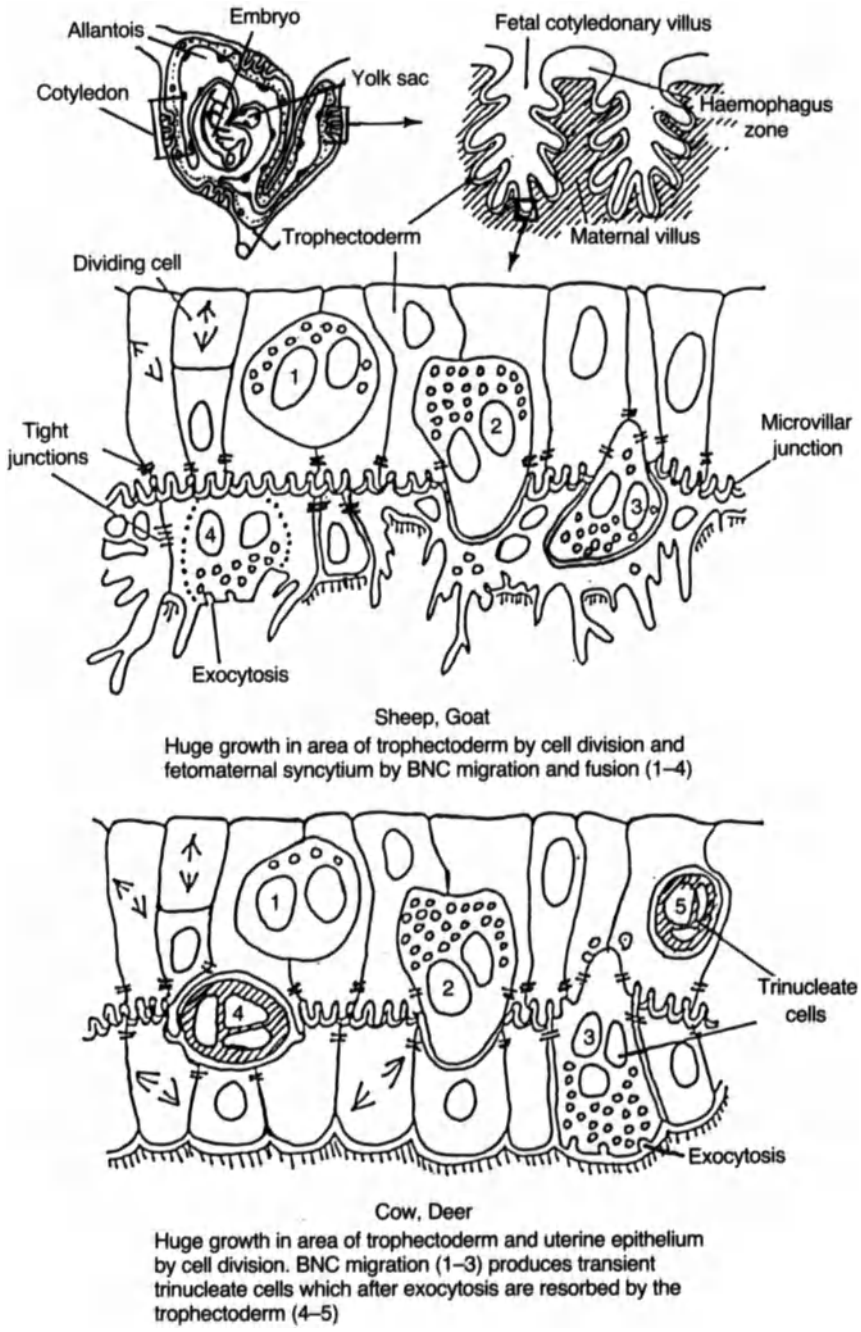


Figure 4.39 The binucleate cell contribution to the definitive ruminant placenta.

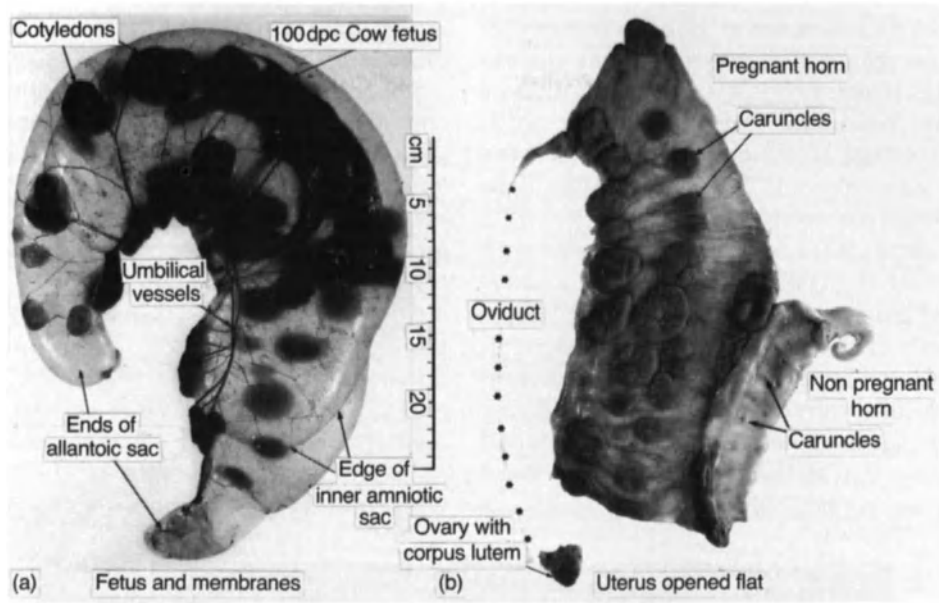


Figure 4.40 Synepitheliochorial placentation. (a) Cow fetus within intact extraembryonic membrane sac(s). Unusually this conceptus was contained within one horn only of the uterus (b), which has been opened out flat. Note the increase in size of the caruncles in the pregnant horn which *in vivo* were enmeshed with the fetal cotyledons. (a and b) 90 dpc, × 0.25.

since if the sheep (normally concave) has some of its caruncles removed surgically from the non-pregnant uterus then the usually adequate resulting placenta has fewer, larger, but now convex, cotyledons (Harding *et al.*, 1985). Hradecky *et al.* (1988) found no consistent correlation between the number or size of cotyledons with the length or degree of branching of the villi in a wide variety of ruminant placentomes.

A logical sequence of villus development can be assembled starting with a completely diffuse epitheliochorial placenta with short stumpy individual villi as in the pig. The microcotyledonary epitheliochorial placenta of the horse is slightly more complex and has small tufts of longer more slender villi. Trophectodermal binucleate cells make a fleeting appearance in the girdle but never assume any permanent structural role in the definitive equid placenta. The sheep polycotyledonary synepitheliochorial placenta has much longer villi more clearly aggregated into discrete placentomes, with characteristic BNCs which play a central role in the formation of the mature villus. The cow has fewer, larger placentomes with more complexly branched villi (Figures 4.40 and 4.41) but the BNCs form only transient fetomaternal minisyncytia. The deer has the most extreme villus aggregation, into 3–8 very large placentomes with villus structure and minisyncytium formation from BNCs very similar to the cow. It is not suggested that this is an evolutionary sequence but it does indicate possible variations of the basic epitheliochorial placenta which exploit the system of villus amplification of the fetomaternal exchange area. Fetomaternal hybrid syncytium formation based on BNCs seems to be central to this villus expansion.

The advantage of concentrating the villi into ever larger placentomes is not obvious but it does result in a considerable increase in the fetomaternal exchange area per gram of delivered fetus. Compact cotyledondary pla-

centas have 5–10 times as much fetomaternal exchange area per gram of fetus as diffuse placentas (Baur, 1977). It is, however, unclear why the ruminant should need so much more exchange area than the horse or if there are any advantages in a few large rather than many small placentomes.

The fetal trophectoderm has microvilli closely interdigitated with those of the uterine epithelium or derived syncytium, forming the characteristic microvillar junction. In the cotyledon local separation at this junction plus a local intermittent vascular leak forms haemophagous zones, large in sheep, small in the cow, in which stagnant maternal blood is apposed to tall columnar trophectoderm which actively phagocytose the red blood cells (Burton, 1982).

There are two major types of synepitheliochorial placenta: the sheep and goat type, with more than 90% of the original caruncular uterine epithelium modified to fetomaternal syncytial plaques; and the cow and deer type, with a similar proportion of (reformed) cellular uterine epithelium but with continuous formation of transient trinucleate minisyncytia (Figure 4.39). In the intercotyledonary areas, after the first third of pregnancy, both types have a cellular uterine epithelium containing characteristic intraepithelial lymphocytes (Lee *et al.*, 1992) but with only occasional minisyncytia produced

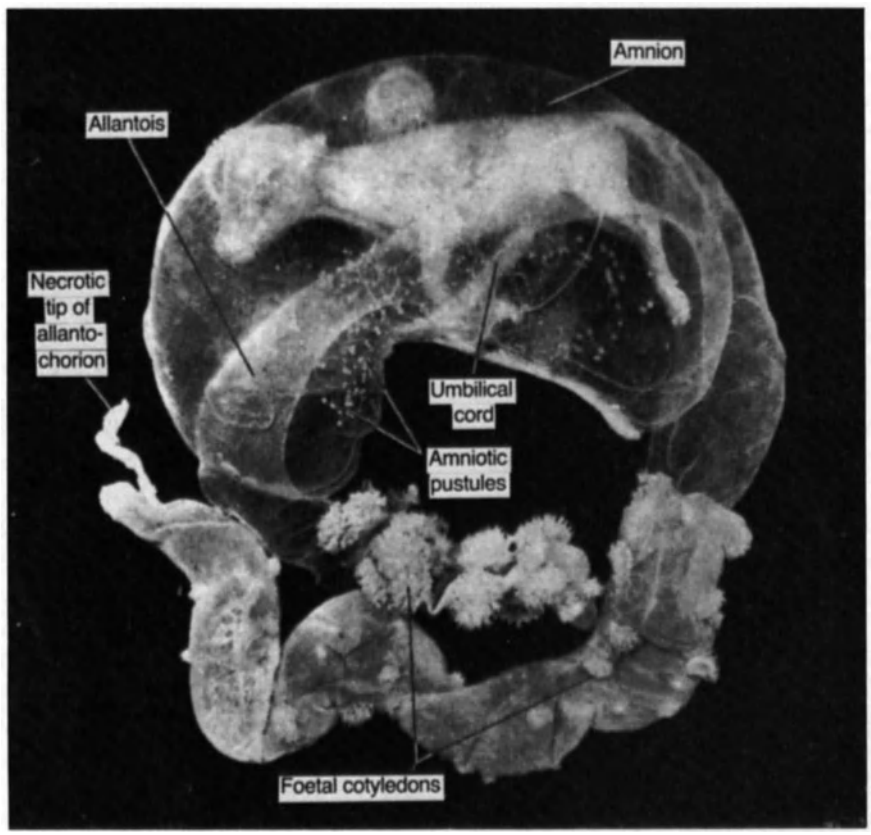


Figure 4.41 Synepitheliochorial placentation. A similar cow conceptus to that in Figure 4.40 but the chorion has been dissected away over the central region to reveal the amnion and the allantois. The villi on the fetal cotyledons on the non-dissected part are well demonstrated; they are clumped into apparently solid masses in Figure 4.40a. 100 dpc, × 0.66.

by BNC migration and fusion (King and Atkinson, 1987). There are small areolae over the mouths of the glands. The percentage of fetomaternal exchange surface contributed by the intercotyledonary area decreases rapidly until at term it is less than 2% of the total in the cow (Baur, 1972).

Development of the sheep and cow placenta will be described as the basic ruminant types.

Sheep, Ovis aries; *cow*, Bos taurus

Oestrous cycle: 16 days, sheep; 21 days, cow
Ovulation: spontaneous
Litter: 1–4
Gestation: about 150 days, sheep, goat; about 280 days, cow, depending on breed
Implantation: superficial; chorionic papillae into glands to immobilize conceptus in sheep and cattle
Amniogenesis: folding
Yolk sac: vascularized by 16 dpc (sheep), 20 dpc (cow) reduced to insignificance by 30 dpc by growth of the allantoic sac.
Chorioallantois: Forms definitive placenta, allantoic sac never surrounds the amnion as in the horse but the allantoic mesodermal vessels vascularize the entire chorion (Figures 4.40 and 4.41).
Shape: Cotyledonary with complex villous structure. The cotyledon number varies from 3 to 150 depending on species.
No decidual reaction
Interhaemal membrane: synepitheliochorial; migratory trophectodermal binucleate cells form fetomaternal hybrid tissue from implantation to term
Accessory placental structures: areolae in intercotyledonary areas; haemophagous zones at bases of fetal cotyledonary villi, larger in sheep than cattle

Most ruminant families share the characteristics above.

Cervidae: deer, moose
Giraffidae
Antilocapridae: pronghorns
Bovidae: cattle, sheep, goats, antelopes, buffalo, bison, gnu

However, the Tragulidae, chevrotains from Java, are reported to have a non-cotyledonary placenta with villi diffusely distributed on the mesometrial side only. BNCs are said to be present in the trophectoderm and the uterine epithelium is probably cellular (Mossman, 1987).

Ruminant fetal membranes The ruminant morula enters the uterus about 4–5 dpc, transforms to a blastocyst, and loses its zona pellucida as it expands two or three times (Rowson and Moor, 1966). One genus (roe deer, Aitken, 1974) arrests the development of the blastocyst (diapause) at this stage prior to implantation. The physiological mechanism is not understood. Bazer and First (1983) provide an excellent summary of the details of uterine secretions, the maternal hormonal levels and structural changes from oocyte to blastocyst in ruminants. Subsequent hormonal changes are detailed in Heap *et al.* (1983).

In the sheep and cow the spherical conceptus comes to rest in a predictable region in the uterus (S.Y. Lee *et al.*, 1977), presumably as a result of myometrial contractions, and immobilizes itself by extending trophectodermal papillae, cellular protrusions, down the glands (Figure 4.38) (Wooding and Staples, 1981; Guillomot and Guay, 1982; Wooding *et al.*, 1982). The conceptus then expands and elongates to fill the uterine lumen (Wintenberger-Torres and Flechon, 1974; Wales and Cuneo, 1989). In the sheep or cow and many other ruminants, if there are two conceptuses each implants about one-third of the length of the uterus up from the cervix, one in each horn of the bicornuate uterus (S.Y. Lee *et al.*, 1977). Single conceptuses implant in a similar position, not always on the side they first enter the uterus, and may eventually expand into both horns. The location of the implantation sites are characterized by local oedema which can be monitored

with i.v. injections of pontamine blue (Boshier, 1970), although no obvious changes can be demonstrated on the two surfaces prior to attachment (Guillomot *et al.*, 1982).

As the blastocyst elongates *in vivo* or *in vitro* it produces considerable amounts of specific proteins (including an interferon from the trophectoderm) which are thought to have important roles both in maintenance of the corpus luteum and immunological defence of the blastocyst (Hansen *et al.*, 1985; Bazer *et al.*, 1986; Fincher *et al.*, 1986; Knickerbocker *et al.*, 1986; Helmer *et al.*, 1987; Sasser and Ruder, 1987; Salamonsen *et al.*, 1988; Bazer *et al.*, 1989; Farin *et al.*, 1989; Ing and Roberts, 1989; Lifsey *et al.*, 1989; Roberts, 1989, 1992; Nephew *et al.*, 1990; Farin *et al.*, 1990; Guillomot *et al.*, 1990; Roberts *et al.*, 1990). In the absence of such trophectodermal proteins, luteolysis is induced by prostaglandins transferred from the uterus by lymphatic and venous countercurrent exchange to the ovarian artery (Heap *et al.*, 1985). Tissue culture studies have recently demonstrated that uterine epithelial cells can be grown (Munson *et al.*, 1990) which can secrete more prostaglandin to the mucosal than the luminal compartment (Cherny and Findlay, 1990).

The yolk sac is fully vascularized by 16 dpc in the sheep but has only a transient existence and is soon displaced by the rapidly elongating allantoic sac. This forms a tubular structure along the full mesometrial aspect of the conceptus by 25 dpc having started as a tiny diverticulum from the hind gut at 16 dpc (Figures 4.1, 4.38, 4.39, 4.40 and 4.41). The allantoic vascularization rapidly spreads over the entire chorion including the extensive amniochorion (Bryden *et al.*, 1972).

Implantation can be defined in ruminants by the start of the BNC migration and fusion with the uterine epithelial cells (16 dpc sheep, 19 dpc goat, 20 dpc cow) (Figures 4.38, 4.42 and 4.43).

No overt endometrial cellular alterations have been reported in any ruminant, and no decidual reaction results from the changes in uterine epithelium from cellular to syncytial (Boshier, 1969; Guillomot *et al.*, 1981; C.E. Smith *et al.*, 1990). However, the uterus grows and the blood flow through the uterine vasculature increases considerably between 11 and 30 dpc (Reynolds and Redmer, 1992). Although the sheep/goat and cow/deer types have significantly different definitive synepitheliochorial placental structure, at implantation BNC migration and fusion produces the same conversion of the caruncular epithelium from cellular to fetomaternal syncytial plaques (Wathes and Wooding, 1980; Wooding, 1984; Wango *et al.*, 1990a,b). At the earliest stage of BNC migration in the sheep, evidence from serial section counting of the number of nuclei within each cellular boundary in the uterine epithelium indicates that possible fusions are restricted. A BNC can fuse with the apex of a single uterine epithelial cell to form a trinucleate cell (TNC) and further BNCs can fuse apically to extend this minisyncytium to five or seven nuclei or beyond. No evidence was found for any minisyncytia with two or four nuclei (Wooding, 1984; Wango *et al.*, 1990a,b). Thus, the syncytia in the uterine epithelium do not result from random lateral fusion of uterine epithelial cells which would produce a continuous range of nuclear numbers per minisyncytium. There is no technical problem in recognizing cells with two nuclei since the same studies clearly confirmed that the trophectoderm consisted of uninucleate cells and BNCs (Wooding, 1984; Wango *et al.*, 1990a,b).

By 20 dpc (sheep) 22 dpc (goat) and 24 dpc (cow) continued BNC migration and fusion coupled with cell death produce syncytial plaques which cover the flat caruncular surface (Leiser, 1975; G.J. King *et al.*, 1979; Wathes and Wooding, 1980; Wooding, 1984; Wango *et al.*, 1990a,b). The maximum number of nuclei per plaque in sheep was estimated to be 24, and the plaques are bounded by tight junctions significantly different from

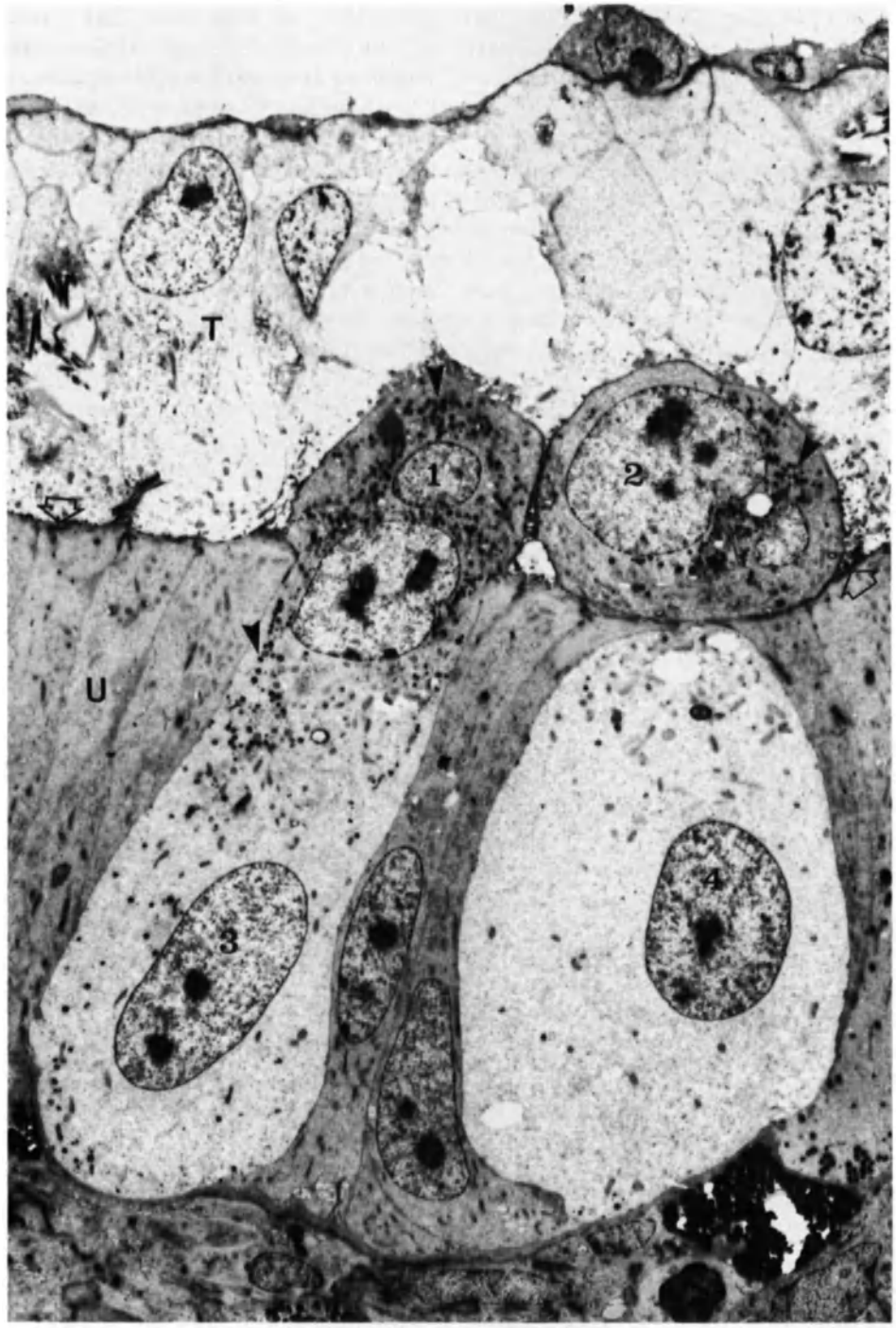
those sealing the uterine epithelial cells (Morgan and Wooding, 1983). The occasional uterine epithelial cell persists also. There are more frequent uninucleate cells remaining in sheep intercaruncular areas but here too, at this early stage, in the sheep and cow syncytial plaques replace the uterine epithelium to a considerable extent (G.J. King *et al.*, 1981; G.J. King and Atkinson, 1987). The intercaruncular uterine epithelium is re-established after 2–3 weeks by rapid division of residual epithelial cells, mostly from gland orifices.

Villus development in the caruncular areas starts at 24 dpc (sheep, goat) and 28–30 dpc (cow) (G.J. King *et al.*, 1979). Clusters of BNCs in the trophectodermal epithelium seem to initiate the process. There is no evidence for any invasion of the caruncular endometrium by the fetomaternal syncytial layer; there seems rather to be a mutual growth of both fetal and maternal tissue. Autoradiographic studies with [^{3}H]thymidine later in pregnancy have produced no evidence of tip growth of villi (Wooding *et al.*, 1981), but in the first half of gestation BNCs are undoubtedly concentrated at the tips of the fetal villi (C.S. Lee *et al.*, 1985, 1986a,b). In the sheep the start of villus formation corresponds to the first synthesis of at least one new protein by the BNCs (C.S. Lee *et al.*, 1985; Morgan *et al.*, 1987). A plausible scenario for villus growth would be the production of an extensive tip syncytium by a high level of BNC migration and fusion. This syncytium is extended into the oedematous endometrial stroma by rapid growth and division of the uninucleate trophectodermal cell layer plus tissue pressure and development of an extensive vasculature in the fetal villus core. These processes would be like inflating a rubber glove into a swelling mound of jelly. Alteration of the endometrium to accommodate this villus growth could well be controlled by a BNC product secreted via the syncytium (C.S. Lee *et al.*, 1985, 1986b; Morgan *et al.*, 1987). The maternal endometrial cells, mainly fibroblasts, are gradually reduced in number as villus development proceeds. The larger maternal blood vessels are eventually surrounded by concentric layers of basement membrane-like material plus fragments, possibly of degenerate maternal endometrial cells or possibly of long slender elongations from the fetal syncytium which extend toward the maternal vasculature (Steven, 1975a,b).

In the sheep and goat, occasionally, between fetal syncytium and maternal vasculature there are large individual cells, probably residual endometrial fibroblasts or pericytes. They have a very uniform structure with a cytoplasm filled with much glycogen, tracts of fibrils and a large deeply divided nucleus (Figure 4.69). They are equipped as if capable of considerable synthesis and secretion having numerous dilated cisterna of rough endoplasmic reticulum, and a sizeable Golgi body with a complement of small dense vesicles. They are present throughout pregnancy. In position and structure they closely resemble a cell type in the endotheliochorial placenta (Figures 4.68 and 4.69).

In the sheep when the villi reach their maximum length at 70–90 dpc there is a considerable volume of fetal connective tissue core (Lawn *et al.*, 1969; Steven, 1983). Subsequently, the surface area of the villi (the fetomaternal hemotrophic exchange surface) grows by ever-increasing subdivision of the initial villi until at term there is very little connective tissue in either fetal or maternal villi. These now consist largely of the two blood vessel systems (Figure 4.44) (see Leiser, 1987) with very little separation because of indentation by the fetal vessels of the trophectodermal epithelium and attenuation of both that and the apposing syncytial plaques.

This is best appreciated on tissue fixed by perfusion via both maternal and fetal arteries (Figure 4.44). Corrosion cast studies of the goat villus vasculature suggest a gross countercurrent flow (Figures 4.45 to 4.48; section 4.2.5c) in the cotyledons.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The approximate 250-μm-long capillary meshworks in the terminal villi (Tsutsumi, 1962) may have a cross-current or a mixed cross and countercurrent microflow (see Figures 4.8, 4.9 and 4.48). The villus vasculature in the cow is more complex than in the goat (Figure 4.49) but the gross and microflow systems are probably very similar.

The increase in the area of the syncytium must be generated solely by BNC migration for no nuclear division has been observed in the syncytium. Quantitative investigations (Wooding, 1983) indicate that BNC migration is maintained at the same high level from implantation to term in all ruminants examined so far, and this is corroborated by the autoradiographic evidence in sheep (Wooding *et al.*, 1981, 1993). These results agree well with Baur's morphometric investigations which show a continuous increase in fetomaternal exchange area virtually to term in the cow and in each of the wide variety of other genera he examined (Baur, 1977). However, there are two reports claiming that the cotyledonary villous surface area in the sheep shows little increase in area in the second half of pregnancy (Stegemann, 1974; Teasdale, 1976). The autoradiographic evidence (Wooding *et al.*, 1981, 1993) indicates that the sheep probably does follow the general pattern of continuous area increase with fetal weight but more work is needed to establish this.

The cow and deer synepitheliochorial placental type forms villi in a similar way but the syncytial plaques formed at implantation are overgrown by rapid division of the residual uterine epithelium (G.J. King *et al.*, 1979). There is still a concentration of BNCs at the forming/advancing tips of the villi (C.S. Lee *et al.*, 1986a) but they form only transient trinucleate cells (TNCs or 'minisyncytia') with the uterine epithelium (Figures 4.60 and 4.61) (Wooding and Wathes, 1980); there is no production of the extensive areas of syncytial plaques seen in the sheep (Wooding, 1984). Plainly the syncytial transformation is not essential for villus growth. Rapid cell division in the uterine epithelium plus the contribution from BNC migration and TNC formation is sufficient to keep pace with the trophectodermal expansion in the cow. This produces an interhaemal membrane which is cellular on both sides as in a pig or horse placenta (Figure 4.39). In deer placentas there have been several reports of more extensive minisyncytia than trinucleate cells in the uterine epithelium, usually toward the tips of the maternal villi (W.J. Hamilton *et al.*, 1960; Kellas, 1966; Sinha *et al.*, 1969) but EM is necessary to investigate the detail of this BNC behaviour. Our limited (unpublished) observations on five deer genera (Chinese water, fallow, muntjac, red and roe) indicate that TNC formation is the standard outcome of BNC migration.

Parturition in the sheep is heralded by a fall in progesterone levels. This is induced by cortisol production and secretion by the fetal adrenal, and this and other hormonal changes (Bazer and First, 1983; Thorburn, 1991) possibly including relaxin are probably instrumental in causing a rapid but specific degeneration of the uninucleate trophectodermal layer (which the BNCs survive) (Perry *et al.*, 1975; Steven, 1975b). There is also an increase in the strength and synchrony of the

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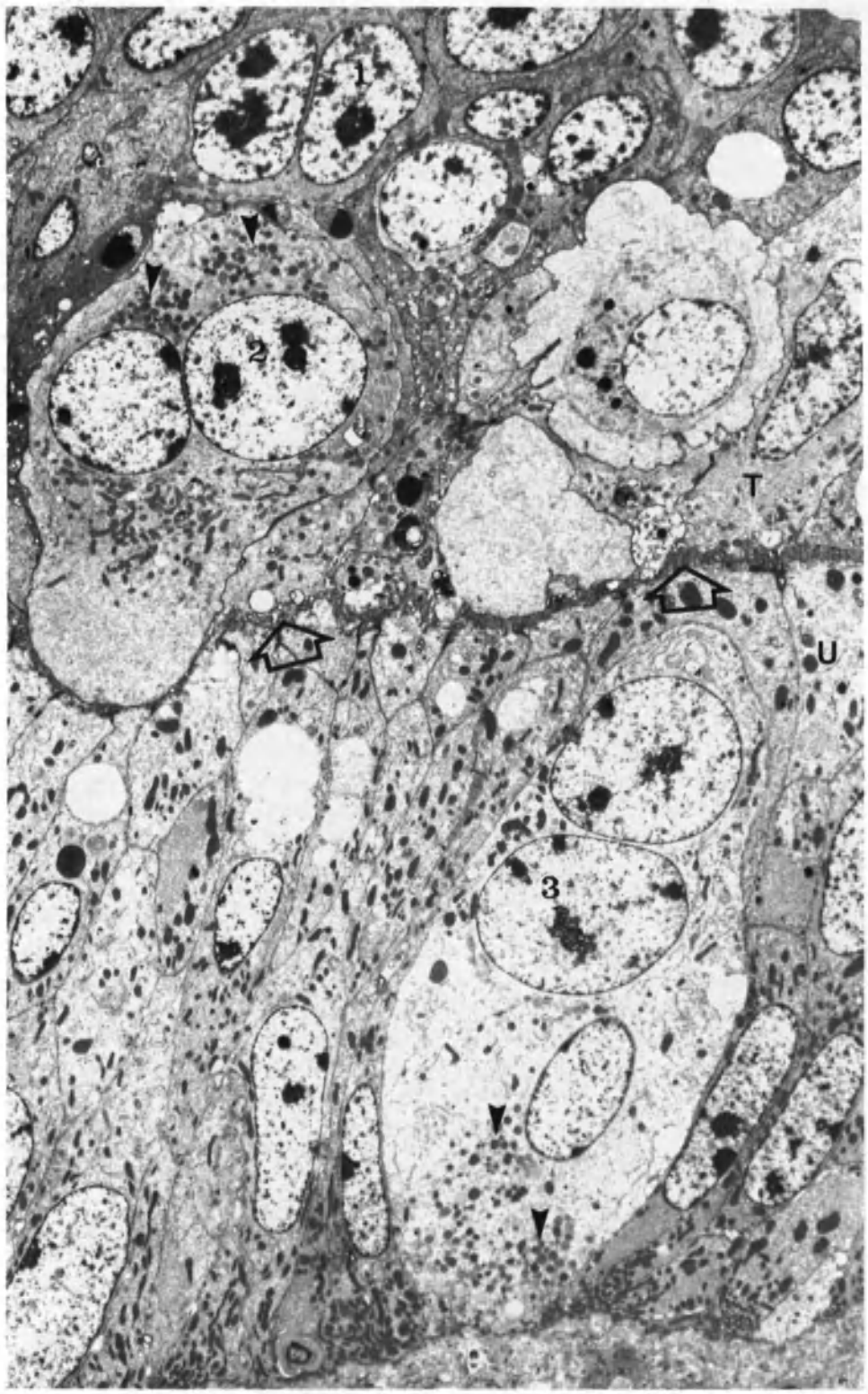
Figure 4.42 Synepitheliochorial placentation. Fusion of two fetal binucleate cells (1 and 2), each with a uterine epithelial cell (3 and 4), at implantation (16 dpc) in the sheep. Cells 2 and 4 were continuous on a different plane of section (see Figure 4.29 and Wooding, 1984). The material is non-osmicated and stained with phosphotungstic acid to emphasize the binucleate cell granules (arrowheads), nuclei and microvillar junction (open arrows), the cytoplasm appears empty. However, after osmium, conventional uranyl acetate and lead section staining produces from the same material micrographs equivalent to those in Figures 4.33 and 4.38. T, fetal trophectoderm; U, uterine epithelium. 16 dpc, × 2500.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myometrial cell contractions due to their considerable hormone-dependent development of gap junctions at this time (Garfield *et al.*, 1979, 1988; Cole and Garfield, 1989) and the cervix softens as a result of increased prostaglandin synthesis (Ledger *et al.*, 1983; Magness *et al.*, 1990). Together with the sudden drop at birth in the fetal placental blood pressure these processes are probably sufficient to produce an efficient means of placental separation within 1–4 h in the sheep and goat. The fetomaternal syncytium remains *in situ* on the maternal villi. As soon as the placenta separates there is a massive infiltration of the maternal caruncular residues by polymorphs and macrophages, which produce a rapid degeneration of the tissue, and the whole mass is sloughed at a level just above the myometrium (Van Wyk *et al.*, 1972). The uterine epithelium is regenerated by cellular overgrowth from the glands at the edges of the caruncular areas (Wagner and Hansel, 1969). In the cow the progesterone level falls and cortisol rises in a similar manner at parturition and relaxin may also be involved (Musah *et al.*, 1987) as well as oxytocin (Fuchs *et al.*, 1992), but there is no degeneration of the trophectodermal layer as in the sheep. Release of the placenta depends upon changes in the molecular forces maintaining the microvillar interdigitation, as in the pig or horse placenta. However, the villi are very much longer than those of the pig or horse and this may be the cause of the significant percentage of cow placentas that do not separate easily (Grunert, 1986). BNCs may also have a role at partus (Gross *et al.*, 1991). A 'retained placenta' is one which has not been delivered 12 h after birth.

Methods for isolating pure populations of BNCs have recently been reported in several laboratories. Such BNCs are as versatile in their synthetic capabilities as they are unique in their developmental and behavioural characteristics. BNCs in short-term primary culture have been reported to produce prostaglandins, progesterone (Hamon *et al.*, 1985; Reimers *et al.*, 1985; Ullman and Reimers, 1989; Wango *et al.*, 1991) and placental lactogens (Rhodes *et al.*, 1986; Morgan *et al.*, 1990). However, all isolated BNC populations contain the complete developmental sequence of BNCs, in our experience usually biased towards the young BNCs with few or no granules. This is quite unlike other isolated exocrine cells such as mammotrophs or somatotrophs from pituitary, which consist almost entirely of mature fully granulated cells. The activities of the isolated BNC population could therefore be characteristic of only one developmental stage, and it is difficult to assess their physiological significance without figures for synthesis of these hormones by the other cell types in the placenta (Shemesh, 1990; Ben David and Shemesh, 1990) which are not yet available. BNCs can be isolated because only they are robust enough to survive the procedure; other, gentler methods will be necessary to separate viable populations of the more fragile uninucleate trophectodermal cells or the syncytial plaques.

In our experience, BNCs will survive in viable condition in primary culture for up to 4 days before rapid deterioration and cell lysis. Only occasional cells adhere to the tissue culture flask. No one has reported nuclear division in BNCs and since they are cells at

Figure 4.43 Synepitheliochorial placentation. Implantation (20 dpc) in the cow. Glutaraldehyde, and osmium fixation. Binucleate cells (1 and 2) with characteristic granules (arrowheads) are migrating up to the microvillar junction (open arrows) between trophectoderm (T) and uterine epithelium (U). The uterine epithelium includes a trinucleate cell (3) with granules (arrowheads) and two round nuclei very similar to those in the binucleate cell. This trinucleate is probably a fetomaternal hybrid cell produced by fetal binucleate cell fusion with a uterine epithelial cell (see Figure 4.29 and Wathes and Wooding, 1980). 20 dpc, × 2800.

the end of a developmental process and contain two nuclei they may be terminally differentiated and so incapable of division. Most studies of isolated BNC have demonstrated **release**, not synthesis, of progesterone prostaglandins or placental lactogen, usually at a declining rate (Reimers *et al.*, 1985; Rhodes *et al.*, 1986; Ullman and Reimers, 1989). Progressive death of the cells and passive release of material seems to be as likely an explanation as normal secretion. This is especially true for placental lactogen since normal BNC development includes migration and fetomaternal fusion prior to release of secretory granules by exocytosis. No one has succeeded in reproducing this behaviour in cultures as yet, but recent work has shown that isolated BNCs can synthesize progesterone from pregnenolone (Wango *et al.*, 1991) and placental lactogen from amino acids (Morgan *et al.*, 1990). Certainly the BNC population has considerable synthetic potential, but how far this contributes to the fetomaternal balance remains to be determined.

Structure and function of placental binucleate cells

Binucleate cells, formed by the absence of cytokinesis after a normal nuclear division, are found occasionally in the placentas of a wide variety of genera. There are two basic types of binucleate cell. The first forms discrete populations, with two 2N nuclei in a cytoplasm of characteristic ultrastructure as in ruminant BNCs (Wooding, 1982b) or equid endometrial cup cells (Allen, 1982). The second is sporadically distributed as part of a continuous spectrum of cells with unusual nuclear forms in a cytoplasm indistinguishable from other cells of the range as in rodent giant trophoblast cells (Pijnenborg *et al.*, 1981). Here the binucleate cell form has been suggested to be either in transition to a single large polyploid nucleus or merely evidence of tardy cytoplasmic division (Brodsky and Uryvaeva, 1977; Ilgren, 1983). One of the characteristic properties of the binucleate cell seems to be a capacity for migration from its site of formation. In ruminants it can pass through tight junctions, in the horse through the uterine epithelium and in hamsters and some other rodents it can invade the uterine arteries as deeply as the myometrial layer.

BNCs are most consistently found and in the greatest number throughout pregnancy, in the trophectoderm of all the ruminants so far studied (Wimsatt, 1951; Wooding, 1982b, 1983). They first appear in the trophectoderm of the blastocyst during the third week of pregnancy (day 15, sheep, Boshier, 1969; Wooding, 1984; day 17, cow, Greenstein *et al.*, 1958; day 18, goat, Wango *et al.*, 1990b). They are found in the fetal trophectoderm apposed to the uterine epithelium at the earliest stages of implantation (day 16, sheep; day 18, goat; day 20, cow), and constitute approximately one-fifth of the total trophectodermal cells for almost the whole of the remainder of pregnancy. There is a decrease in the last week of pregnancy in the sheep and goat (Table 4.3).

These generalizations apply to both singleton and multiple pregnancies (Wooding, 1983).

There is no evidence for any specialized stem cell; binucleate cells can originate by division of any uninucleate cell in the trophectodermal epithelium. The division produces two cells, one of which has no apical tight junction. The nucleus of this cell divides again without subsequent cytokinesis, thereby forming a binucleate cell. This binucleate cell rapidly releases any contact with the basement membrane. It therefore starts and usually completes its maturation process with only rare tiny desmosomal-like contacts with the uninucleate cells of the epithelium; no tight or gap junctions have been observed (Lawn *et al.*, 1969; Boshier and Holloway, 1977). There is one published method for growing this epithelium with included BNCs

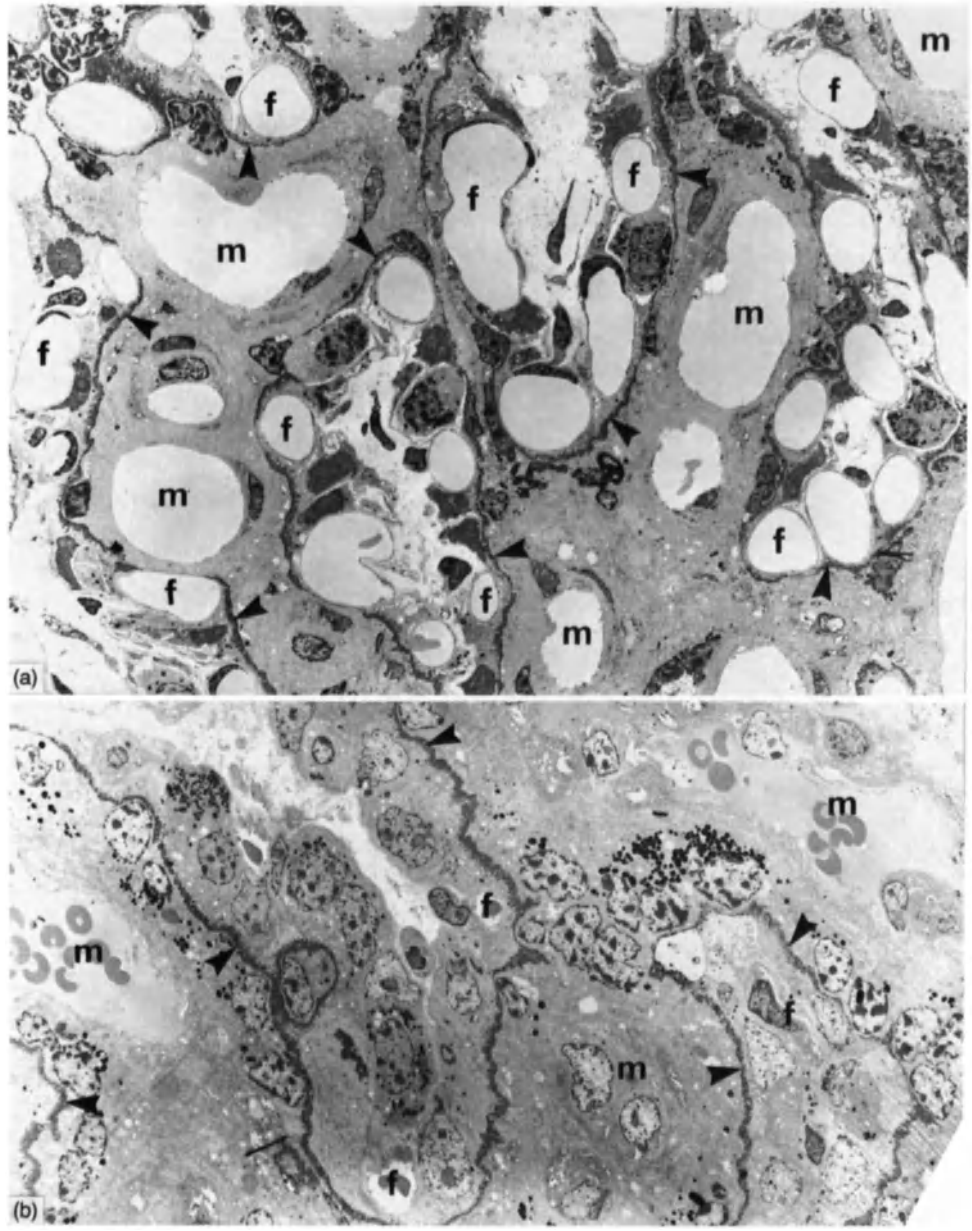


Figure 4.44 Synepitheliochorial sheep placenta. Vascular relationships. Electron micrographs of sections of cotyledons at a similar gestational age (a) perfused with fixative through both maternal and fetal blood vessels (b) fixed by dicing up fresh cotyledon in the same fixative used in (a). Only by the use of double perfusion can an accurate idea be obtained of the relative sizes and dispositions of the blood vessels *in vivo*. The result emphasizes the concept that the placenta should be considered primarily as a close apposition of blood circulations, not a mass of tissue through which blood circulates. Phosphotungstic acid staining on non-osmicated araldite embedded tissue. Arrowheads, microvillar junction (which is **not** the fetomaternal junction, the arrowheads are all on top of the fetomaternal hybrid syncytial layer see section 4.3.6b); f, fetal capillaries; m, maternal capillaries. (a) 130 dpc, × 1300. (b) 128 dpc, × 1300.

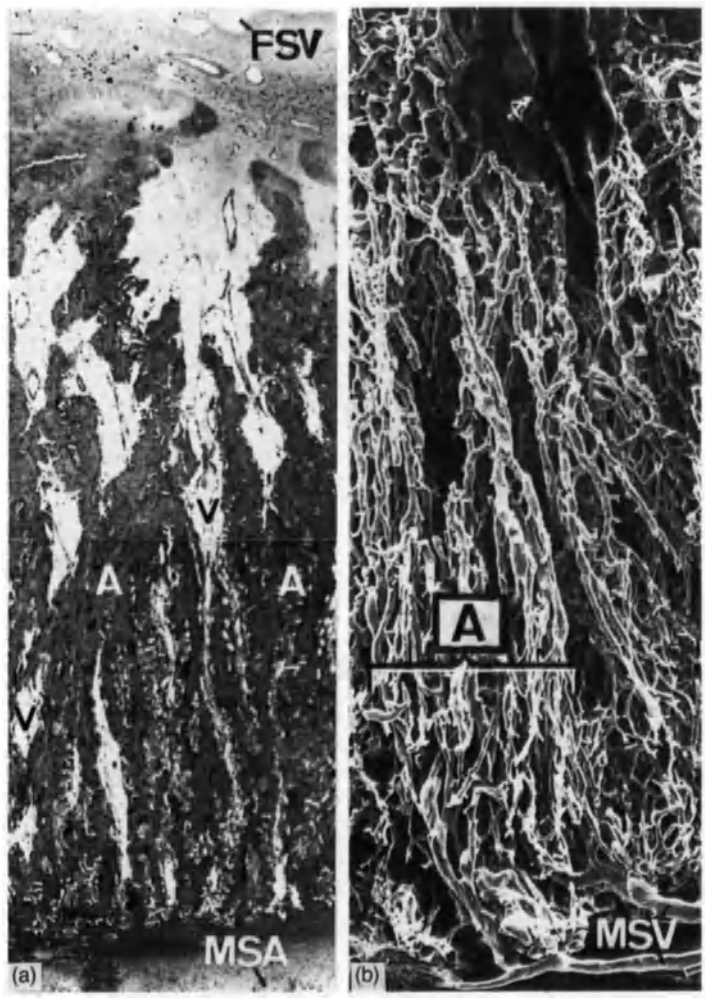


Figure 4.45 Synepitheliochorial placentation. Vascular relationships in the goat cotyledon. (a) Light micrograph of a transverse section through a cotyledon showing the fetal villi (V) in the maternal crypts (A) 120 dpc, × 35. (b) Scanning electron micrograph of a vascular corrosion cast showing the maternal capillary meshwork. This links the maternal stem artery MSA (not present on this preparation but which delivers oxygenated blood to the region corresponding to the top of the micrograph) to the maternal stem veins (MSV) at the bottom, which return the blood to the uterine vein. In (b) the maternal network corresponding to the region A in the section is similarly marked. FSV, fetal stem vein. 120 dpc, × 50. (From Leiser, 1987.)

in tissue culture (Steven *et al.*, 1980b). However, we and others have been unable to repeat this work, and although there are more recent methods of growing uninucleate, polarized, trophectodermal epithelium (Ralph *et al.*, 1989; C.S. Lee *et al.*, 1990) no BNCs could be identified in these cultures.

The young binucleate cell has a few mitochondria in a dense, ribosome-filled cytoplasm with no glycogen (Figure 4.50).

It rapidly develops an extensive system of swollen rough endoplasmic reticulum cisternae and a very large Golgi apparatus. The Golgi produces large numbers of dense gran-

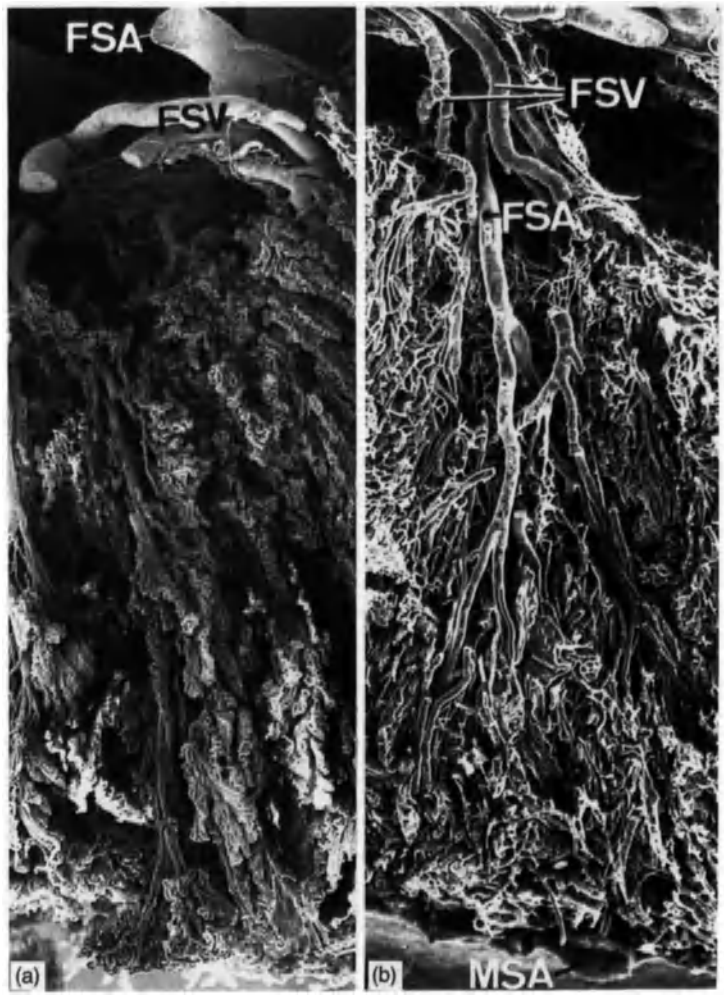


Figure 4.46 Synepitheliochorial placentation. Scanning electron micrographs of corrosion casts of (a) the fetal vasculature and (b) both fetal and maternal blood vessels. The detailed architecture of the blood vessels is well demonstrated, with the fetal capillaries much finer than the maternal. The penetration of the fetal stem arteries (FSA) direct to the tips of the villi is clear in (b). FSA, MSA, fetal or maternal stem arteries; FSV, fetal stem veins. (a and b) 120 dpc, × 35. (From Leiser, 1987.)

ules and the cell grows very large (Figure 4.51).

In the first third of pregnancy in sheep and goat a fully granulated 'mature' binucleate cell frequently also contains a large lipid droplet and/or a membrane-bound crystalline inclusion which are typical of the surrounding uninucleate cells, emphasizing the derivation of one from the other. All the binucleate cells in the trophectoderm can be arranged in a single developmental sequence (Wooding, 1982a,b, 1983, 1984); there is no clear structural evidence for two populations as suggested by Boshier and Holloway (1977).

BNCs from all species so far examined contain a unique structural element, the double lamellar body (DLB). This is a discrete small

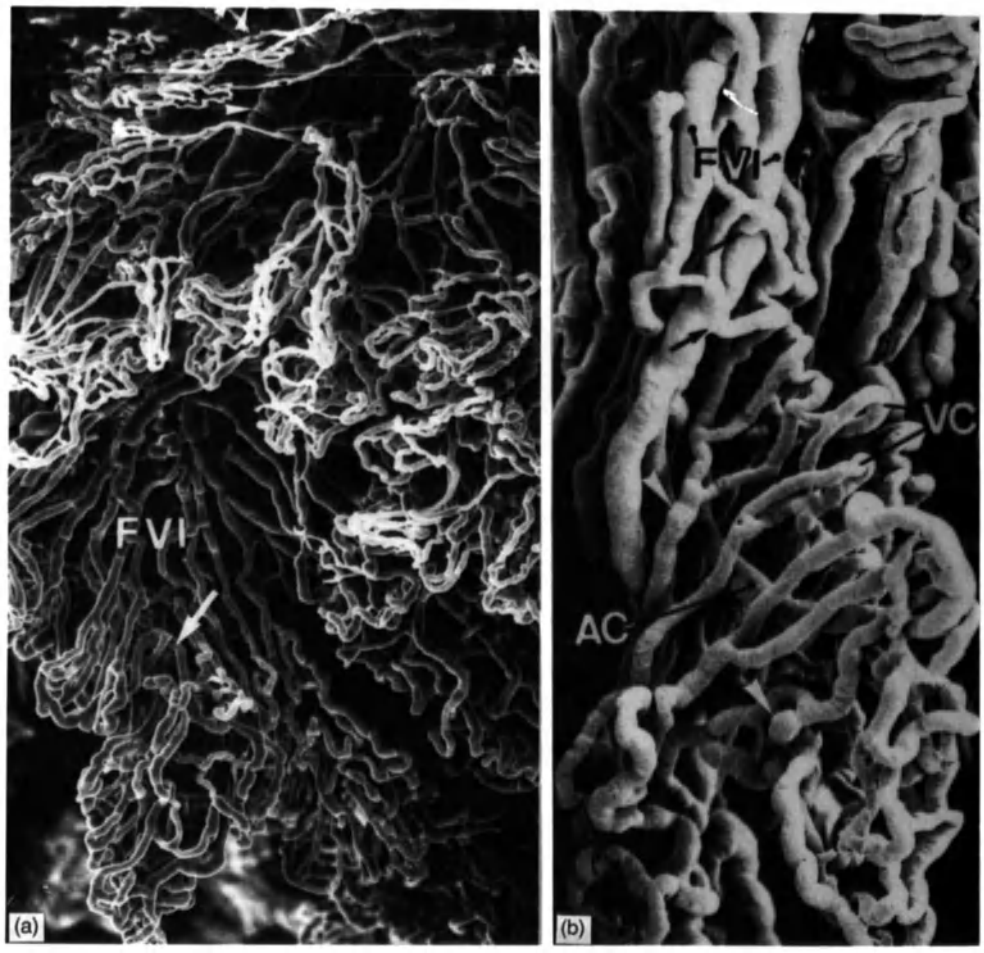


Figure 4.47 Synepitheliochorial placentation. (a) Corrosion cast of the capillaries forming the tips of the goat fetal villi (asterisks). Some villi are connected in series (white arrow, see Figure 4.48). 133 dpc, × 170. (b) Villus tip capillaries showing the central supply arteriole (AC) and venous connections to the next villus (VC). White arrowheads: sinusoidal widenings. 133 dpc, × 330. (From Leiser, 1987.)

area of double membranes of characteristic branching structure intermittently continuous with endoplasmic reticulum cisternae and usually located near the Golgi body (Figures 4.52 to 4.55).

In a recently divided BNC the double membranes have numerous associated small clear vesicles (Figures 4.52 and 4.54). DLBs in mature migrating BNCs lack these clear vesicles as do DLBs in the fetomaternal syncytia, which we consider to be derived from the migration (Figures 4.53 and 4.55).

The DLB thus shows a clear developmental sequence but as yet its function is unknown. Autoradiographic (Wooding *et al.*, 1981), histochemical (Wooding, 1980) and immunogold EM (C.S. Lee *et al.*, 1986b–d) studies show no involvement of the DLB in protein synthesis, placental lactogen storage, phosphatase localization or BNC granule production.

The mature binucleate cell lies very close to the microvillar junction but normally does not form part of the tight junction between

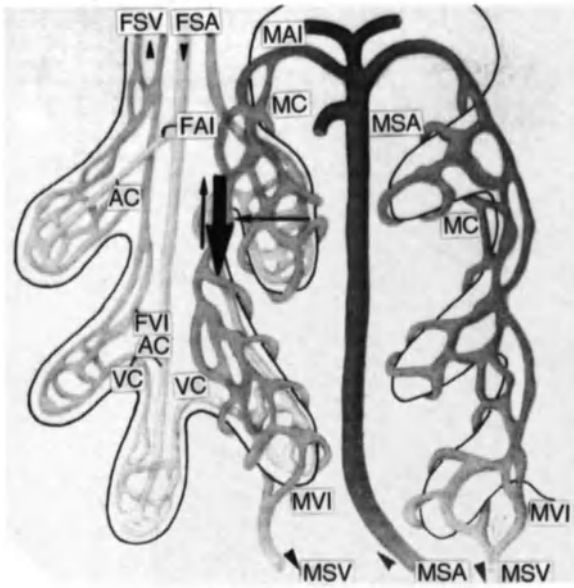
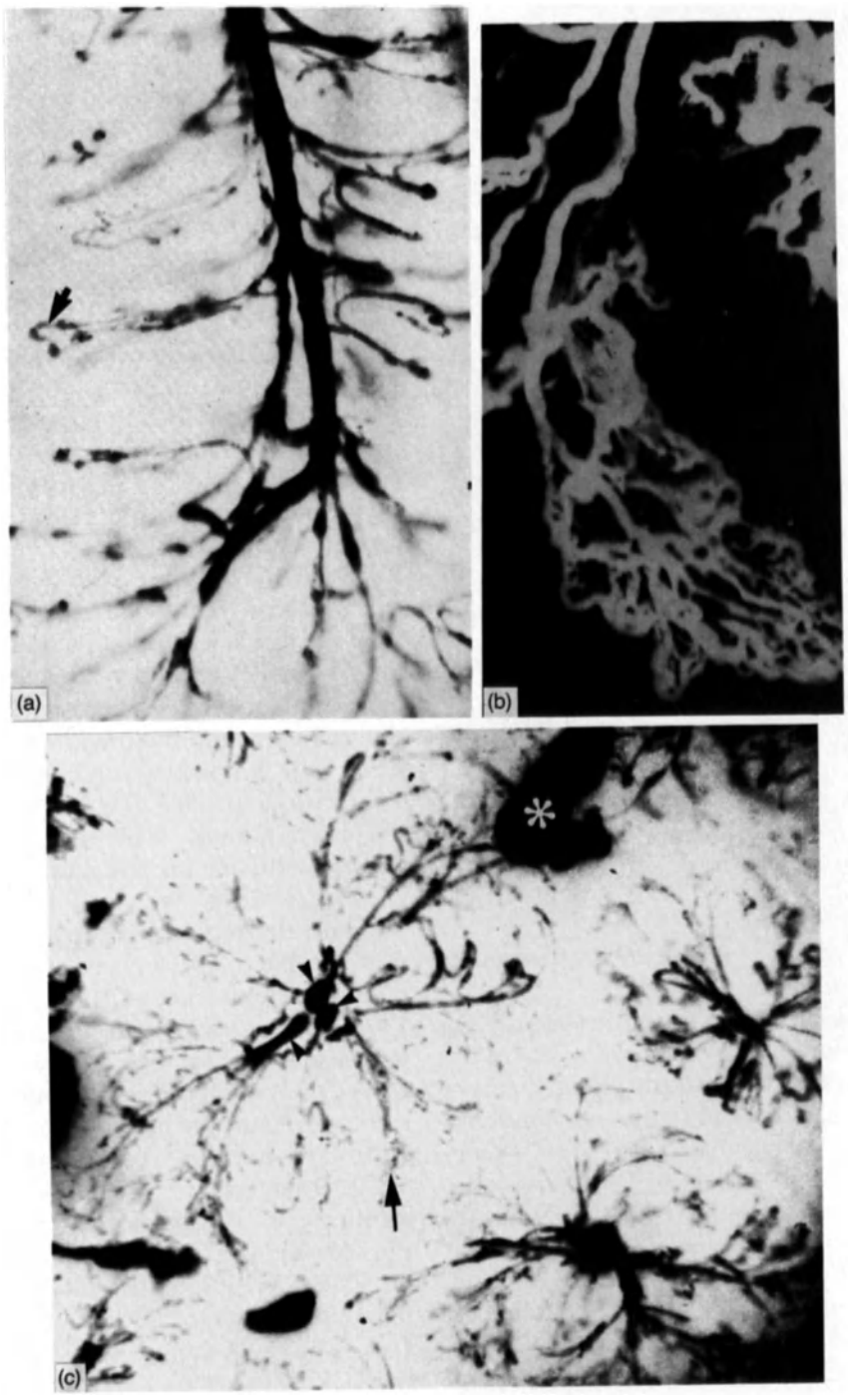


Figure 4.48 Synepitheliochorial placentation. Leiser's (1987) schematic summary of his corrosion study of goat placental vasculature. Both fetal and maternal stem arteries (FSA, MSA) run directly to opposite sides of the placenta, ensuring a grossly counter-current flow. However, the details of the arteriolar distributions are said to indicate a cross-current flow at that level. From the stem arteries (FSA, MSA) the arteriolar supply (FAI or MAI) to each villus tip may be direct (AC) or in series, derived from an adjacent villus (VC, MC). (From Leiser, 1987.)

the apices of the uninucleate trophectodermal cells. Migration through this tight junction is the next stage in the binucleate cell life cycle. Throughout pregnancy 15 – 20% of the total population of binucleate cells are part of this tight junction in all four genera enumerated so far (cow, deer, goat and sheep) (Wooding, 1983; Table 4.3). Only mature binucleate cells are found with tight junction structures and micrographs of these cells at all stages of passage through the tight junction (Figures 4.56, 4.58 and 4.59) together with autoradiographic (Wooding *et al.*, 1981) and freeze-fracture (Morgan and Wooding, 1983) evidence (see below) provides a clear indication that these cells are migrating.

This migration starts at implantation and in the goat, sheep and cow produces a syncytium bordering the maternal tissue by fusion of the migrated binucleate cells with some individual uterine epithelial cells plus an elimination of the remainder (Wathes and Wooding, 1980; Wooding, 1984) (Figures 4.38, 4.39, 4.42 and 4.43). In the sheep and goat cotyledon this syncytium persists throughout pregnancy (Lawn *et al.*, 1969) (Figures 4.58 and 4.59). In the cow at implantation there is a transient formation of syncytium by migrated binucleate cells fusing with uterine epithelial cells but only a partial loss of the uterine epithelium. This bovine syncytium is then eliminated by displacement by continuing division of the remaining uterine epithelial cells (G.J. King *et al.*, 1979; Wathes and Wooding, 1980). Subsequently, during the remainder of pregnancy in cow and deer, the migrated binucleate cell fuses with an individual cell derived from the original uterine epithelium to form a transient trinucleate cell (Figures 4.60 and 4.61) which dies after the granules from the original binucleate cell have been released (Wooding and Wathes, 1980; Wooding, 1987) (Figure 4.39).

This migration was suggested by the earliest workers on the ruminant placenta (Assheton, 1906; Wimsatt, 1951), but subsequent electron microscopic investigations were unable to find any supporting evidence (Bjorkman and Bloom, 1957; Lawn *et al.*, 1969; Bjorkman 1968,1970). A study of sheep binucleate cells in 1977, for example, concluded that there were 'no published, well resolved pictures of binucleate cells in contact with the syncytium' (Boshier and Holloway, 1977). By developing a method for selective staining of non-osmicated material with phosphotungstic acid to emphasize only the binucleate cell granules and the microvillar junction (Figures 4.56 to 4.61) (Wooding, 1980) and by subsequent use of ultrastructural (Wooding *et al.*, 1980) and immunocytochemical (Figure 4.57) (Wooding, 1981; C.S. Lee *et al.*, 1986b–d) techniques it has now


(a)
(b)
(c)

been possible to demonstrate clearly both the fact and extent of the migration.

The frequency of migration in the sheep and goat needs to be sufficient to maintain the syncytium, in which no nuclear division has ever been demonstrated, throughout the enormous expansion in area which occurs during formation of the cotyledonary villi. It is clear that static micrographs can never provide direct evidence for a dynamic process. However, injection of radioactive thymidine into fetal sheep or goats and subsequent autoradiography of the fetomaternal interface layers shows that labelled nuclei are initially found exclusively in the uninucleate trophectoderm, subsequently in mature binucleate cells and finally in the syncytium (Wooding *et al.*, 1981, 1993). There was no evidence for a population of binucleate cells which became labelled but did not migrate.

In the goat and sheep cotyledon, maintenance of the substance of the syncytium would seem to be an important function for migration. However, in the intercotyledonary regions in goat and sheep the trophectoderm is apposed to a cellular uterine epithelial derivative, as it is in the whole of the cow and deer placenta, yet binucleate migration still occurs. It has been suggested that the primary function of the migration is to deliver the binucleate cell granules to the plasmalemma bounding the maternal connective tissue (Steven *et al.*, 1978). Exocytosis would then deliver the granule contents close to the maternal circulation, and there is good ultrastructural evidence for such a process from sheep and goat syncytium and cow trinucleate cells (Wooding, 1987; Wango *et al.*, 1990a).

Ovine and bovine placental lactogens (oPL and bPL) have been localized exclusively in the BNC granules and Golgi body and the granules in the syncytium by electron microscopic immunocytochemistry (Figure 4.57). No label was found over the Golgi bodies in the syncytium, indicating that synthesis and packaging of BNC granule contents occurs before BNC migration (Wooding, 1981; C.S. Lee *et al.*, 1986b–d; Wooding and Beckers, 1987). This agrees with the hypothesis of delivery of the granules to the syncytium by binucleate cell migration in order to evade the barrier to diffusion of protein molecules like placental lactogen (PL) provided by the tight junctions sealing the trophectoderm. Placental lactogens have so far been localized only in binucleate cells in the sheep by immunocytochemistry (Martal *et al.*, 1977; Watkins and Reddy, 1980; Wooding, 1981; C. S. Lee *et al.*, 1986b–d; Morgan *et al.*, 1987) and cow (Verstegen *et al.*, 1985; Duello *et al.*, 1986; Wooding and Beckers, 1987). The only claims for localization of oPL to uninucleate chorion cells (Carnegie *et al.*, 1982; Chan *et al.*, 1990) are far less convincing than any of the other studies cited here (see Wooding *et al.*, 1992). Biochemical organelle isolation techniques have also shown that placental lactogens copurify with a granule fraction (oPL, Rice and Thorburn, 1986; bPL, Byatt *et al*, 1986). More recently, hybridization histochemical studies (Milosavljevic *et al.*, 1989; Kappes *et al.*, 1992) have shown that PL mRNA is found only in ovine and bovine BNCs. There are other mol-

Figure 4.49 Synepitheliochorial cow placenta. Vascular structures. The injected and sectioned material (a and c) and the latex cast (b) demonstrate the complexity of the villus structure in the cotyledon. (a) Longitudinal and (c) transverse sections of villi demonstrate the delicacy and detail of the capillaries at the ends of the side branches (arrows). This can also be appreciated in the latex cast (b). There seem to be three vessels in the core of the fetal villus (b, arrowheads), presumably stem arteries and veins. In (c) the largest vessels (asterisks) are probably maternal veins. (From Tsutsumi, 1962.) (a) 105 dpc, × 100. (b) 90 dpc, × 100. (c) 105 dpc, × 90.

Table 4.3 Binucleate cell numbers throughout pregnancy in ruminants*

	Stage of gestation days post coitum	*Percentage of BNC in trophectoderm*	*Percentage of BNC population migrating***
A. SHEEP			
	12–15 (isolated preimplantation conceptuses)	0–3	28
	16–29	18	24
	41–67	20	21
	70–105	18	26
	114–139	17	18
	142–147 (term=145)	8	20
B. GOATS			
	27–90	21	21
	95–130	21	23
	135–148	16	25
C. COWS			
	18–28	20	25
	37–260	21	12
D. DEER			
	Implantation	16	12
	Implantation to 60	18	15

*For details of breeds, counting methods, statistical limits see Wooding (1983).
**A migrating BNC is defined as one which is part of the trophectodermal apical tight junction.

ecules, including glycoproteins, present in high concentrations in the binucleate cell granules as shown by immunogold studies (for example, one glycoprotein is defined so far only by its monoclonal antibody SBU-3) (C.S. Lee *et al.*, 1985, 1986b–d; Figure 4.57b) and by their selective staining with periodic acid–Schiff at light microscope level (Wimsatt, 1951; Lee *et al.*, 1985, 1986a) and phosphotungstic acid at electron microscope level (Wooding, 1980; Wooding and Wathes, 1980). This staining is found in cow, deer, goat and sheep binucleate cells.

Another constituent recently claimed for the BNC granules is the acidic 78-kD 'pregnancy-specific protein B' (PSPB or bPAG) (Sasser *et al.*, 1989; Zoli *et al.*, 1992). This is found in the maternal blood of all pregnant ruminants tested so far, but it persists longer post partum than would be expected if it is produced solely in the BNCs (Humblot *et al.*, 1990). There is evidence that, although all BNCs appear similar ultrastructurally, the content of their granules may depend on the location of the BNCs or the age of the conceptus. Ovine intercaruncular BNCs do not express oPL or SBU-3 (C.S. Lee *et al.*, 1985, 1986c,d), and although oPL is present in caruncular BNC in conceptuses from 16 dpc SBU-3 is not found until 24 dpc (Morgan *et al.*, 1987).

Binucleate cell migration, therefore, provides the fetus with a general method of delivering large molecules to the maternal circulation. However, in the sheep, oPL is present in both maternal and fetal circulations and if all binucleate cells migrate to the maternal side it is difficult to understand

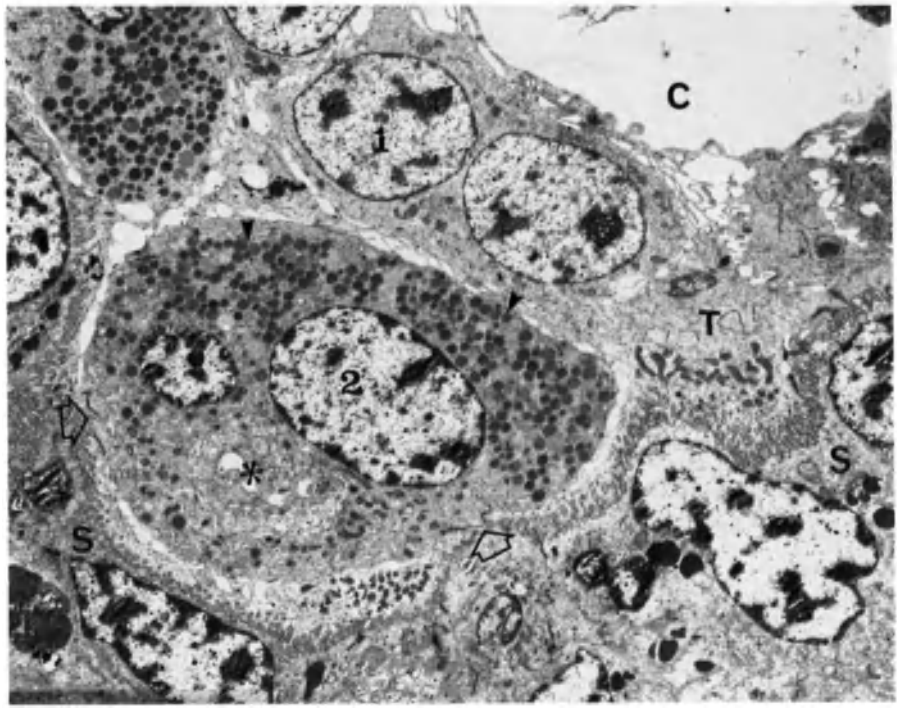


Figure 4.50 Synepitheliochorial placentation. Glutaraldehyde/osmium fixation. Development of fetal binucleate cells (1, young; 2, mature) in the trophectoderm (T) of the definitive placenta of the goat. Note the numerous characteristic granules (arrowheads) and large Golgi body (asterisk) in the mature binucleate cell, which has started to migrate up to the microvillar junction at two points (open arrows). S, fetomaternal syncytial layer; C, fetal connective tissue. 127 dpc, × 2500.

the origin of the fetal serum oPL (Chan *et al.*, 1978; Martal and Lacroix, 1978). What little evidence there is indicates that there is no transfer of oPL from maternal to fetal circulation (Grandis and Handwerger, 1983) or from fetus to mother (Schonecht *et al.*, 1992). This problem is more extreme in the cow, in which the bPL concentration in the maternal circulation is always very much lower than on the fetal side (Beckers *et al.*, 1982; Wallace *et al.*, 1985). Despite this, all the bPL-containing BNCs appear to migrate and bPL-containing granules are found only in BNCs and their derivatives (Wooding and Beckers, 1987). Even allowing for the vastly greater maternal blood volume, the amount reaching the fetal circulation is very significant from the earliest stage measured.

There is no evidence in cow or sheep for a subpopulation of BNCs which degranulate on the fetal side, but there may be a constant low level of release of granules prior to BNC migration, which would be very difficult to detect ultrastructurally. Alternatively, there may be a constitutive pathway for oPL and bPL secretion independent of the conventional regulated exocytotic mechanism (Burgess and Kelly, 1987). This is an important point because exogenously administered oPL elicits biological responses in the fetal sheep (Battista *et al.*, 1990) consistent with earlier suggestions (A.W. Bell, 1984; Gluckman *et al.*, 1987) that oPL may serve as an important somatotrophic hormone in the fetus.

This presence of placental lactogens in both fetal and maternal circulations is in sharp

contrast with the restriction of equine chorionic gonadotropin (eCG) produced by the horse endometrial cup BNCs entirely to the maternal circulation (Ginther, 1979). In this case the BNCs do not mature (i.e. produce eCG-containing granules) until they are in transit to the cup site in the maternal endometrium (section 4.3.6a).

It has been suggested that binucleate cell migration in the sheep can be increased by fetal pituitary stalk section or adrenalectomy, but this was based on the assumption that migration was insignificant in normal pregnancy (Barnes *et al.*, 1976; Bass *et al.*, 1977; Steven *et al.*, 1978; Lowe *et al.*, 1979). Recent quantitative studies have shown in the sheep that there is no significant difference between the BNC migration level in normal pregnancy and that found after a variety of experimental procedures including pituitary stalk section, adrenalectomy, carunclectomy, hypophysectomy and administration of progesterone synthesis inhibitors and EGF (Wooding *et al.*, 1986). The production, maturation and migration rate of the BNCs seem to be totally unaffected by administration of this wide variety of compounds and procedures, as are the oPL blood concentrations (Taylor *et al.*, 1982, 1983). Those agents which initiate the complex hormonal and structural changes leading to parturition do decrease BNC production (not migration) (Wooding, 1982a,b, 1983), but it is not yet possible to identify what factor(s) it is that controls BNC production. During implantation the earliest evidence for binucleate cell formation in sheep (Boshier, 1969) is found at the same time as the first demonstration of placental lactogen in the blastocyst of the sheep (Martal and Djiane, 1977; Morgan *et al.*, 1987) or cow (Flint *et al.*, 1979). In the last week of pregnancy there is a fall in binucleate cell num-

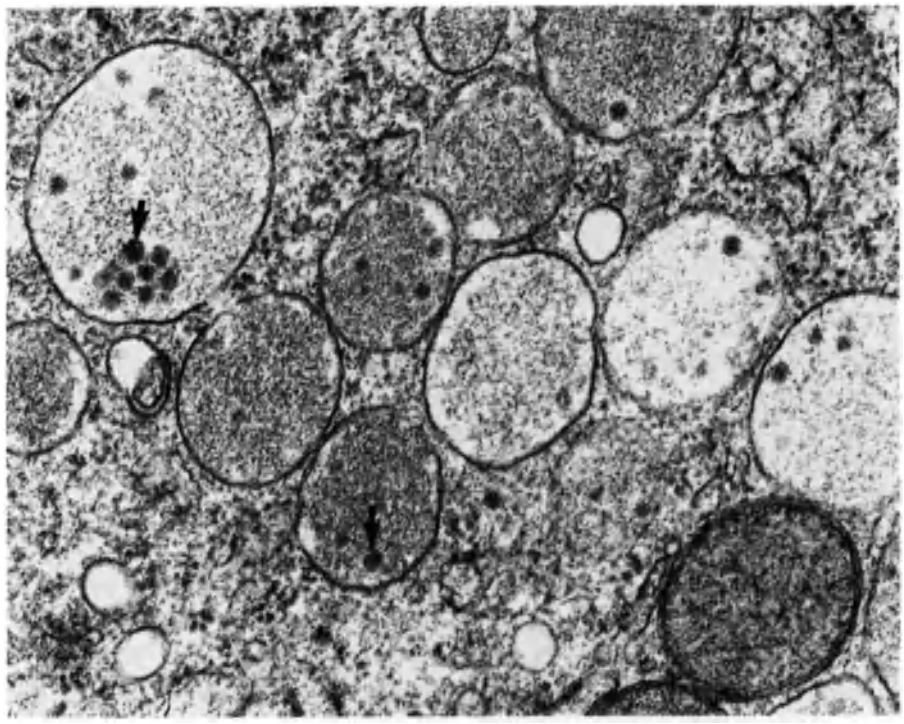


Figure 4.51 Synepitheliochorial placentation. Glutaraldehyde/osmium fixation. Detail from cow binucleate cell showing granules containing characteristic microvesicles (arrows). 49 dpc, × 46 000.

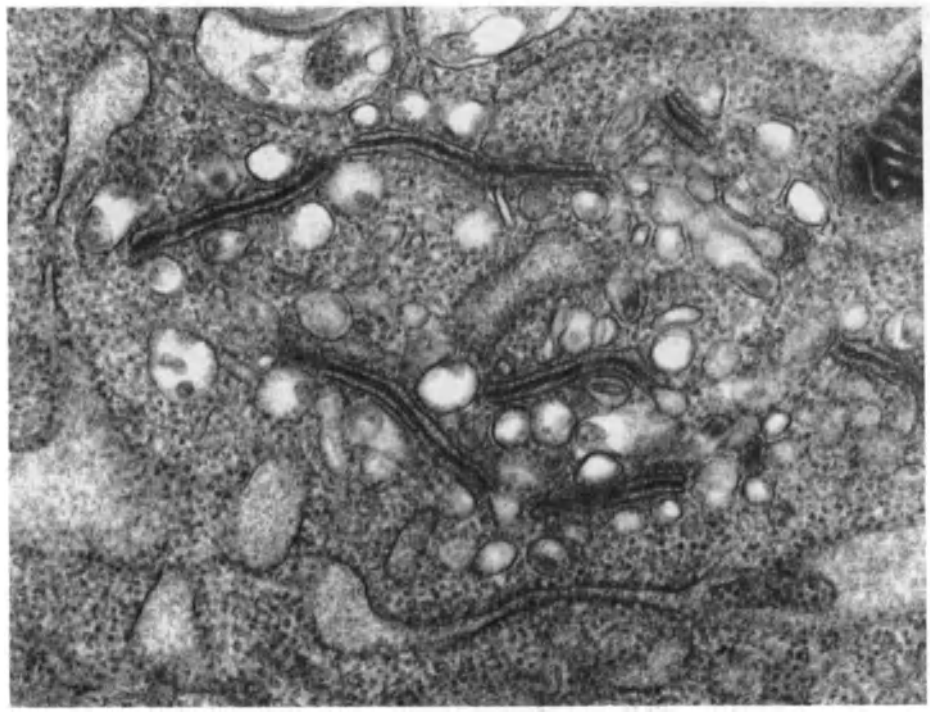


Figure 4.52 Synepitheliochorial sheep placenta (a) The 'double lamellar body' (DLB), characteristic of ruminant binucleate cells, in a young binucleate cell, note the frequent electron lucent vesicles. 114 dpc, × 50 000.

bers and this correlates with a similar fall in oPL concentration in both fetal and maternal circulations (Martal and Djiane, 1977). However, until it is possible to predictably manipulate the oPL concentration, there is insufficient evidence to assume that the blood concentrations are directly related to binucleate cell numbers. It could be that it is the rate of migration of the cells or exocytosis of the granules which is the primary locus of control of release of oPL into the circulation. Interpretation of recent *in vitro* experiments on the control of oPL secretion is difficult because of the complexity of the system. oPL release could originate from unmigrated BNCs (via the presumed route into the fetal circulation) or from granules delivered to the syncytium by BNC migration – the normal route into the maternal circulation. The latter is an order of magnitude greater than the former *in vivo*. Secretion from placentomal slices (said to involve protein kinase C, Battista *et al.*, 1990) will presumably involve both routes, whereas isolated trophectodermal uninucleate and binucleate cells (Burke *et al.*, 1989) will be restricted to only the fetally directed secretion.

There is now a considerable amount of evidence that all ruminant fetal binucleate cells migrate towards the maternal tissues, presumably to deliver the content of their granules to the maternal circulation. At least one hormone is involved in the sheep, goat and cow. The importance of this particular fetal contribution to the establishment and maintenance of pregnancy remains to be established, but there is an increasing amount of evidence that the placental lactogens can modify the maternal metabolism to favour fetal nutrition and growth (Munro, 1980,

1986; Waters *et al.*, 1985; Freemark and Handwerger, 1986; Freemark *et al.*, 1987; Thordarson *et al.*, 1987; Chêne *et al*, 1988; Forsyth, 1991; Handwerger, 1991; Byatt *et al.*, 1992).

(c) Endotheliochorial placentation (dog, cat, bat)

In endotheliochorial placentation no uterine epithelium survives implantation (Figure 4.5). The cellular chorioallantoic trophectodermal cells produce, by division and fusion, a fetal syncytium apposed to an amorphous layer around the persisting maternal endothelium. The amorphous layer (also referred to as the interstitial layer or, misleadingly, 'membrane') is probably produced by the syncytium and is equivalent, ultrastructurally and in molecular constituents, to the basement membrane of the maternal endothelium. It is neither uniformly thick nor continuous; processes of the fetal syncytium penetrate through it to reach a close relationship with the maternal endothelium in places, and it includes occasional large residual endometrial cells. This structure is characteristic of the early and definitive endotheliochorial placentas which are zonary (Figure 4.62).

The zones are made up of grossly parallel fetal villi, which are labyrinthine in architecture. Usually, but not always, there is a large haemophagous zone at the edge or in the centre of the zonary structure. Outside the main zone of the placenta a cellular fetal trophectoderm is loosely apposed to a persistent uterine epithelium and there are small areolae over the gland mouths. Endotheliochorial placentation is characteristic of carni-

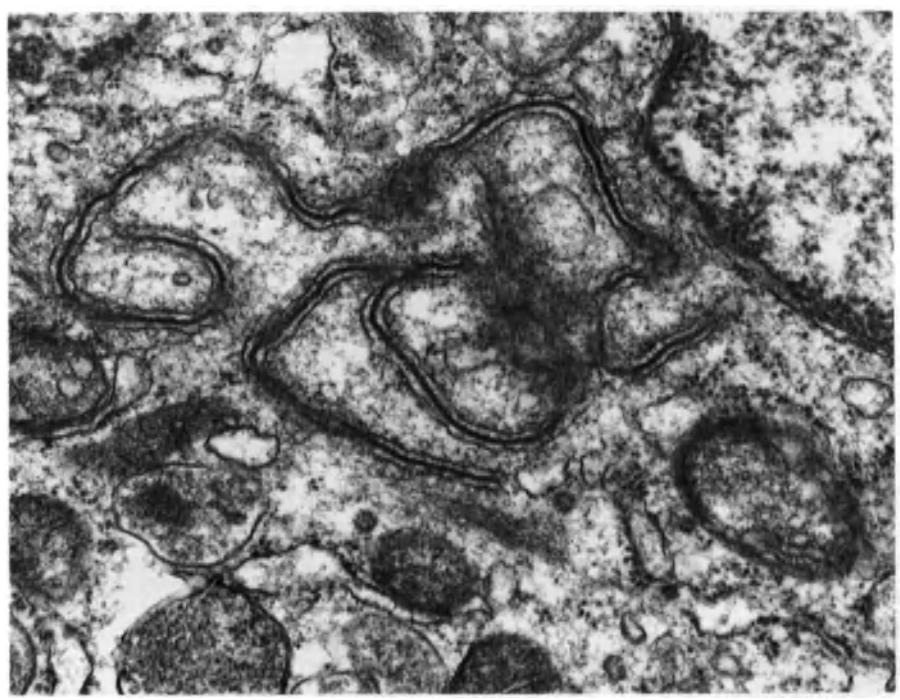


Figure 4.53 Synepitheliochorial sheep placenta. Mature DLB (see Figure 4.52) from the fetomaternal hybrid syncytium which is formed in part from mature binucleate cell migration and fusion, shows no associated vesicles such as those seen in the immature form (Figure 4.54). 114 dpc, × 40 000.

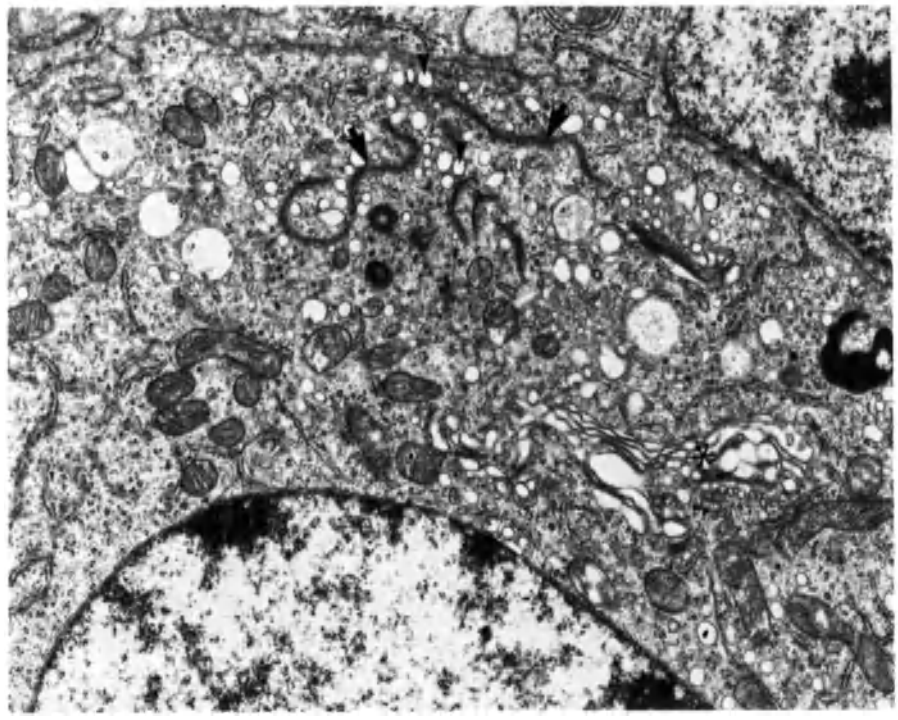


Figure 4.54 Synepitheliochorial cow placenta. The double lamellar body (arrows) has electron lucent vesicles (arrowheads) associated with it in the young non granulated binucleate cell, but there are very few in the mature granulated cell (Figure 4.55) 49 dpc, × 12 000.

vores and many bats but is also found in individual genera in other orders – Proboscidea (elephant), Tubulidentata (aardvarks), Rodentia (*Dipodomys*, the kangaroo rat), Insectivora (*Talpa* the European mole, and *Blarina*, the shrew) and also *Bradypodida* (the sloths) (Mossman, 1987).

The cat and dog will be taken as the characteristic examples of this placental type.

Dog, *Canis familiaris*; cat, *Felis domestica*

Oestrous cycle:
dog, one 21-day cycle during the single yearly breeding period
cat, 15–21-day cycles; several during each of the breeding periods which occur 2–3 times per year
Ovulation: Spontaneous in the dog, induced by coitus in the cat. For endocrinological details during pregnancy see Concannon *et al.* (1989).
Litter: 3–6
Gestation: 55–65 days
Implantation: superficial; central, antimesometrial
Amniogenesis: folding
Yolk sac: well-vascularized choriovitelline placenta up to 24 dpc with non-vascularized short villi of endotheliochorial structure (Figure 4.62). The yolk sac is then separated from the chorion by growth of the allantoic sac but persists to term (Figure 4.62) as a highly vascularized collapsed sac with a characteristic ultrastructure (S.Y. Lee *et al.*, 1983).
Chorioallantois: forms definitive placenta, the allantoic sac surrounds and vascularizes the amnion and chorion
Shape: zonary

Decidua: very infrequent modification of stromal cells in the endometrium just below the invading tips of the villi

Cats, and to a lesser extent dogs, have large cells, probably of endometrial stromal origin, throughout the placental lamellae between the trophectodermal syncytium and the maternal endothelial cells. Most authors refer to them as decidual cells but nothing is known of their function (Figure 4.68) (Wislocki and Dempsey, 1946; J.W. Anderson, 1969; Malassine, 1974). Ruminants have similar cells in an equivalent position (see Figure 4.69).

Interhaemal membrane: endotheliochorial

Accessory placental structures: areolae in paraplacental region. Haemophagous zones at the margin or centre of the zonary placenta (Figure 4.65)

Fetal membranes and placental development The newly pregnant bicornuate uterus has a uniform development of numerous glands around the lumen (Wislocki and Dempsey, 1946; Barrau *et al.*, 1975). In the cat and dog, blastocysts enter the uterus at 5 dpc and establish an even spacing by 8 dpc, at which time they are sufficiently large to distend the uterus. If only two blastocysts are present one implants in each horn about halfway up (Amoroso, 1952). There is evidence for up to 40% transuterine migration between horns in cat and dog (Shimizu *et al.*, 1990).

In several animals with endotheliochorial placentas, such as bears, badgers and seals, the blastocyst can be held in diapause and implantation delayed for several months by physiological mechanisms which are still poorly understood (Enders and Given, 1977; Mead, 1993). Once the blastocyst has

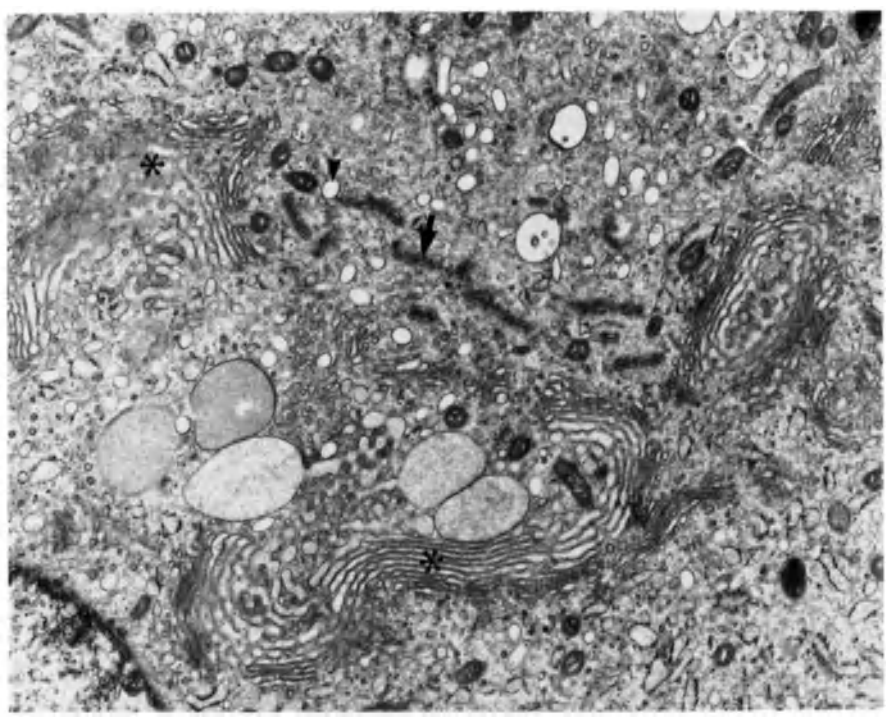


Figure 4.55 Synepitheliochorial cow placenta. The mature granulated binucleate cell has a double lamellar body (arrow) with few associated vesicles. Note the large size of the Golgi body (asterisks) in the mature cell. 130 dpc, × 12 000.

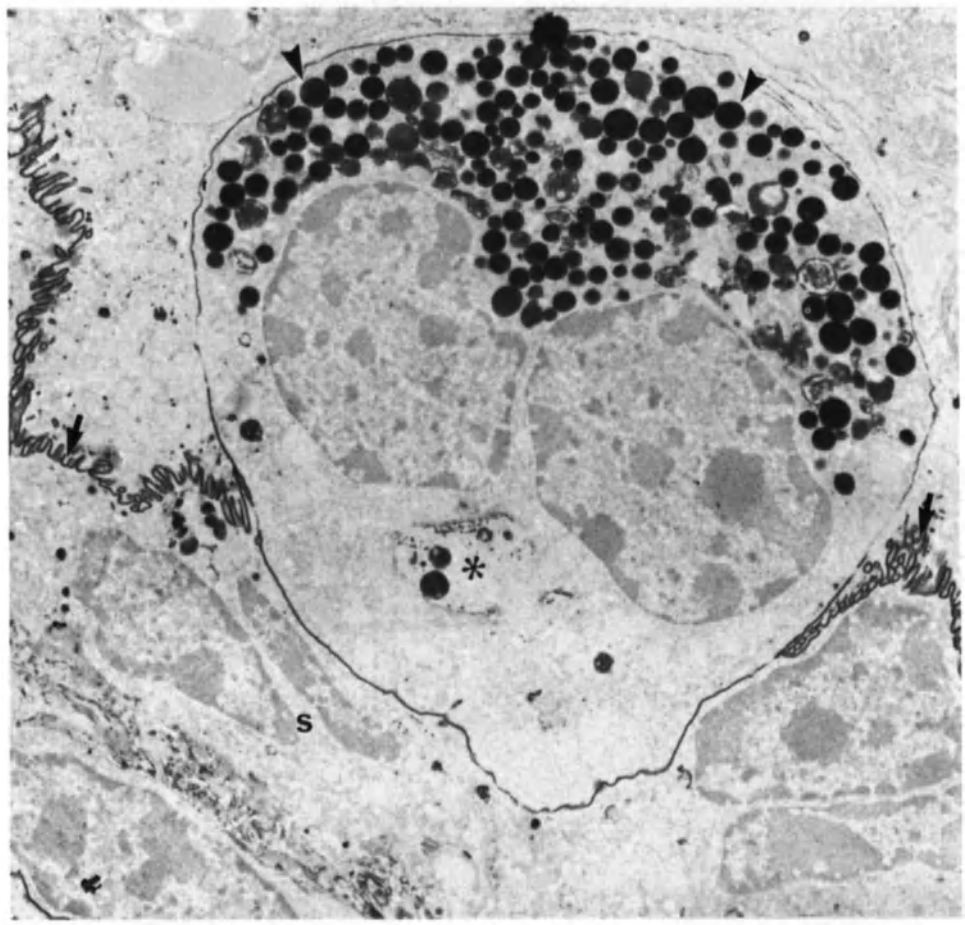


Figure 4.56 Synepitheliochorial sheep placenta. Phosphotungstic acid staining. Binucleate cells migrate across the microvillar junction (arrows) throughout pregnancy carrying their characteristic granules (arrowheads), formed mostly before migration in the Golgi body (asterisk), into the fetomaternal syncytium (S). 114 dpc, × 5000.

expanded to distend the uterus (central implantation) the yolk sac is vascularized over most of its mesometrial half, and a functional trilaminar choriovitelline placenta is formed (Figures 4.62 and 4.63).

In the cat, cellular contact and subsequent interdigitation of microvilli between trophectoderm and uterine epithelium starts on 13 dpc. Around this time full apical junction complexes have been observed (Leiser, 1979) between cells from the two epithelia, but it is not clear whether these are formed as a result of fetal trophectoderm intrusion between, or fusion with, the cells of the uterine epithelium. The uterine epithelium undergoes sporadic lateral fusions and a considerable amount of individual cell death. At this stage the cellular trophectoderm (cytotrophoblast) shows numerous divisions and produces a syncytium inextricably enmeshed with remnants of the cellular and newly syncytial uterine epithelium (Leiser, 1979). The fetal syncytiotrophoblast is actively phagocytic, rapidly displacing and replacing the uterine

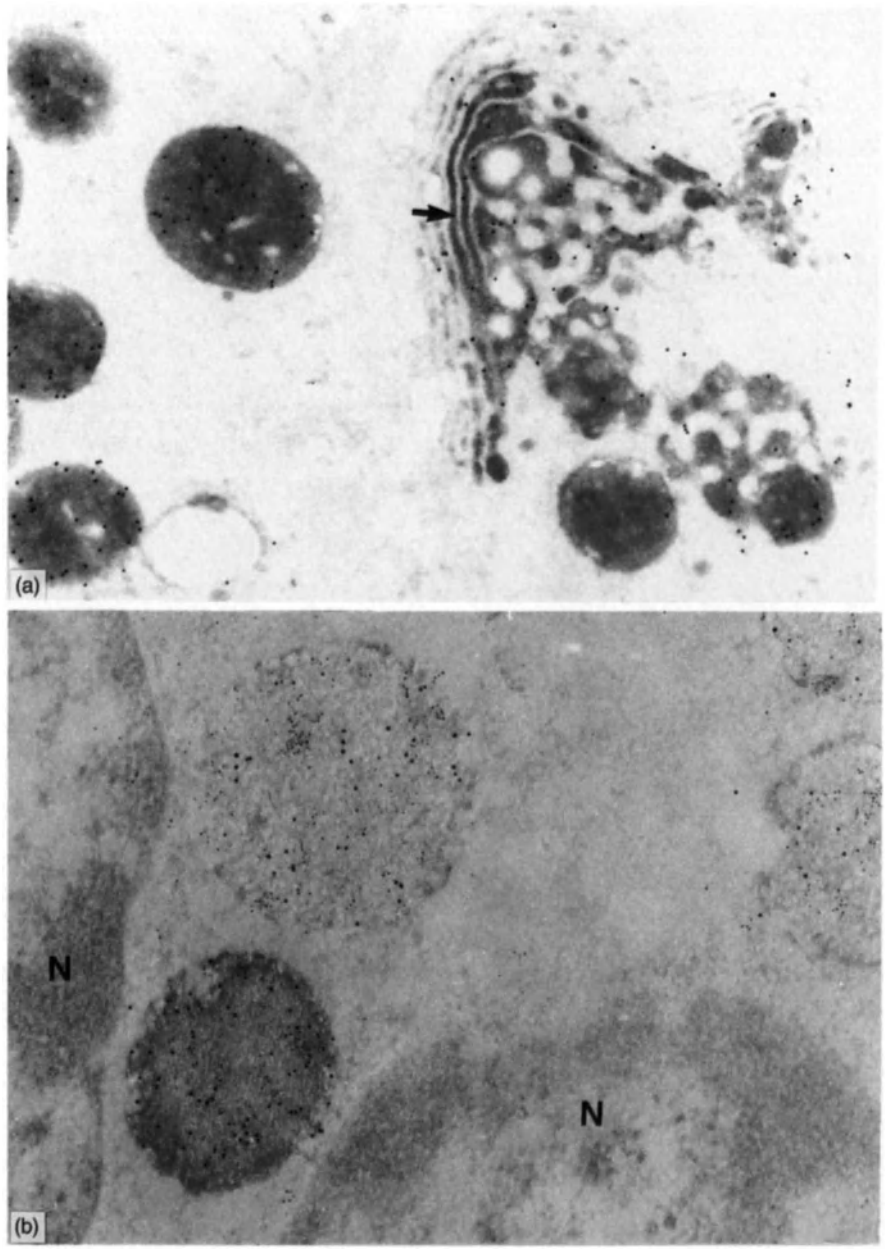


Figure 4.57 Synepitheliochorial sheep placenta. (a) Binucleate cell Golgi body immunostained for ovine placental lactogen. The gold particles localize the lactogen exclusively to the *trans* cisternae of the Golgi stack (arrow) and the granules. 114 dpc, × 38 000. (b) Colocalization of ovine placental lactogen (5 nm gold) and the SBU-3 antigen (10 nm gold) on the binucleate cell-derived granules in the placental syncytium. N, nucleus. (From Lee *et al.*, 1986c). 114 dpc, × 40 000.

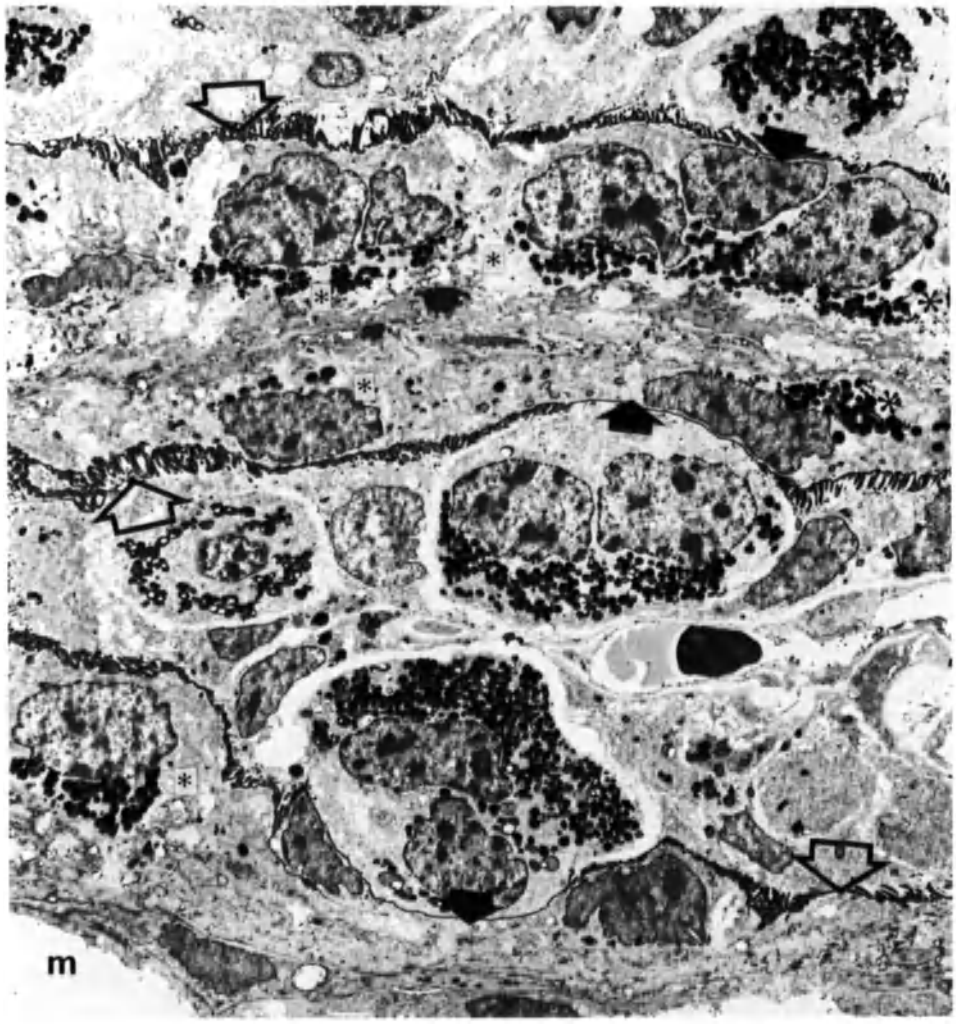


Figure 4.58 Synepitheliochorial sheep placenta. Binucleate cells migrate (solid arrows) across the microvillar junction (open arrows) throughout pregnancy (Figure 4.58, 65 dpc; Figure 4.59, 142 dpc) delivering their granules to the base of the fetomaternal syncytium (asterisks). There are usually many granules in the syncytium at early stages (Figure 4.58) of pregnancy, few are found near term (Figure 4.59). m, maternal blood vessels (see Figure 4.39 and Wooding, 1981). 65 dpc, × 2600.

epithelium (Barrau *et al.*, 1975; Leiser, 1979). It then flows around the maternal capillaries and establishes the endotheliochorial pattern of the interhaemal membrane. The absorption of the maternal uterine and glandular epithelium and the continued erosion of the connective tissue are achieved by swelling masses of syncytiotrophoblast. This is produced by the rapid proliferation of the cytotrophoblast and forms short avascular villi which make up the trilaminar choriovitelline placenta (Figure 4.63) (Amoroso, 1952). These villi extend into the endometrium all over the conceptus, except for the area immediately around the embryo, which has only just completed amnion formation by

fusion of head and tail folds. After the amnion is formed, the allantoic outgrowth from the hind gut rapidly expands into the exocoelomic space around and below the embryo. Fusing with the trophectoderm it forms a chorioallantoic placenta, displacing the yolk sac and its mesodermal vascularization over the whole of the spherical conceptus (Figure 4.62). The allantoic vascularization is much more aggressive than that of the yolk sac and pushes rapidly into the villi. This development of fetal blood vessels plus the prolific generation of syncytiotrophoblast from cytotrophoblast forms the basis of the rapid advance of these villi down the glands, stripping away all gland epithelium (which first transforms into a syncytium) and connective tissue. At the same time the maternal vasculature is expanding and elongating. As Mossman (1987) has pointed out, formation of the zonary placental villi cannot just be based on erosion by the fetal syncytium and its advance into the endometrium, the final length of the villi is much too long for this. There has to be considerable expansion of the maternal blood capillary system. This point can be best appreciated by examination of a developmental series of transverse sections through the cat placenta (Figure 4.63 and 4.64).

The structure of the initial interhaemal membrane is endotheliochorial, as it is at term. Indentation of the fetal cyto- or syncytiotrophoblast by the fetal capillaries is considerable towards the end of pregnancy, and there is usually a definite attenuation of the amorphous (interstitial) layer (J.W. Anderson, 1969; Barrau *et al.*, 1975). The maternal endothelium varies considerably in thickness depending upon the species. In the bear it is typically three to four times thicker than in seals, but the functional significance of the differences is obscure (Amoroso, 1952; Sinha and Erickson, 1974; Wimsatt, 1974).

The area of the interhaemal membrane increases and its thickness decreases as the

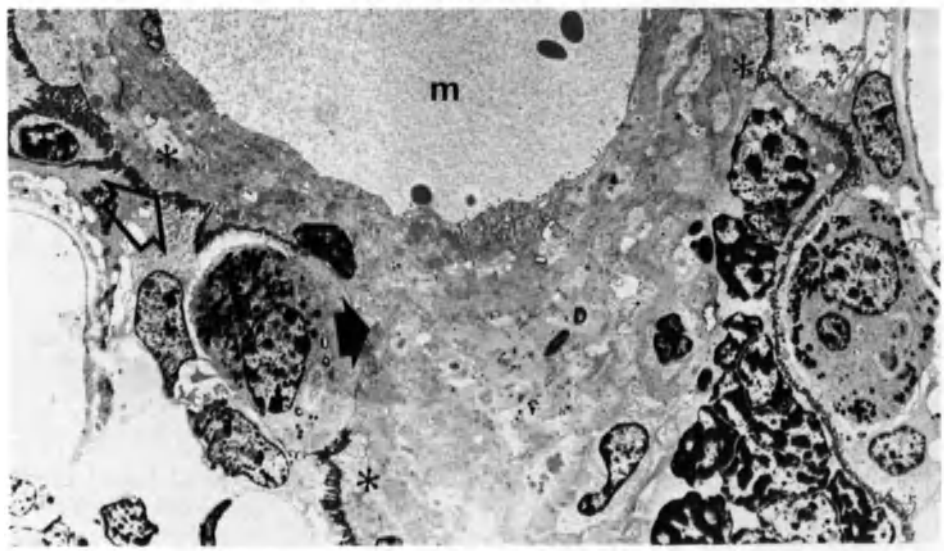


Figure 4.59 Synepitheliochorial sheep placenta. Binucleate cells migrate across the placental microvillar junction (open arrows) right up to term (145 dpc). The micrograph shows a binucleate cell (solid arrow) at day 142 dpc migrating into the fetomaternal syncytium (asterisks) which contains fewer binucleate cell derived granules than at earlier stages (see Figure 4.58). 142 dpc, × 1400.

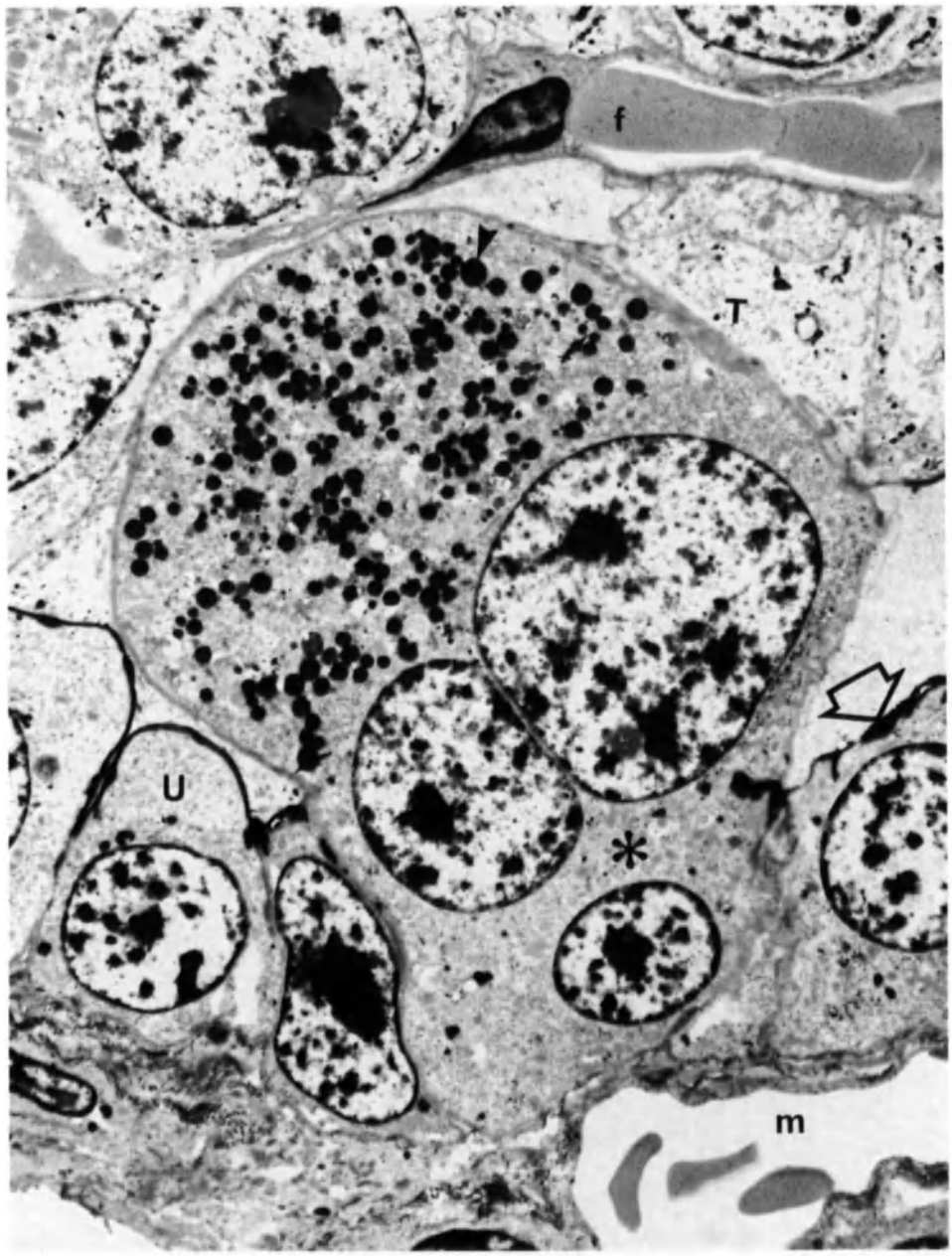


Figure 4.60 Synepitheliochorial cow placenta. Throughout pregnancy binucleate cells (B) migrate from the fetal trophectoderm (T) across the microvillar junction (open arrow) to fuse with uterine epithelial cells (U) producing trinucleate cells (asterisks). These eventually release their granules (arrowheads) close to the maternal blood vessels (m), die and are resorbed by the trophectoderm (see Figure 4.39 and Wooding and Wathes, 1980) f, fetal blood vessel. 70 dpc, × 4400.

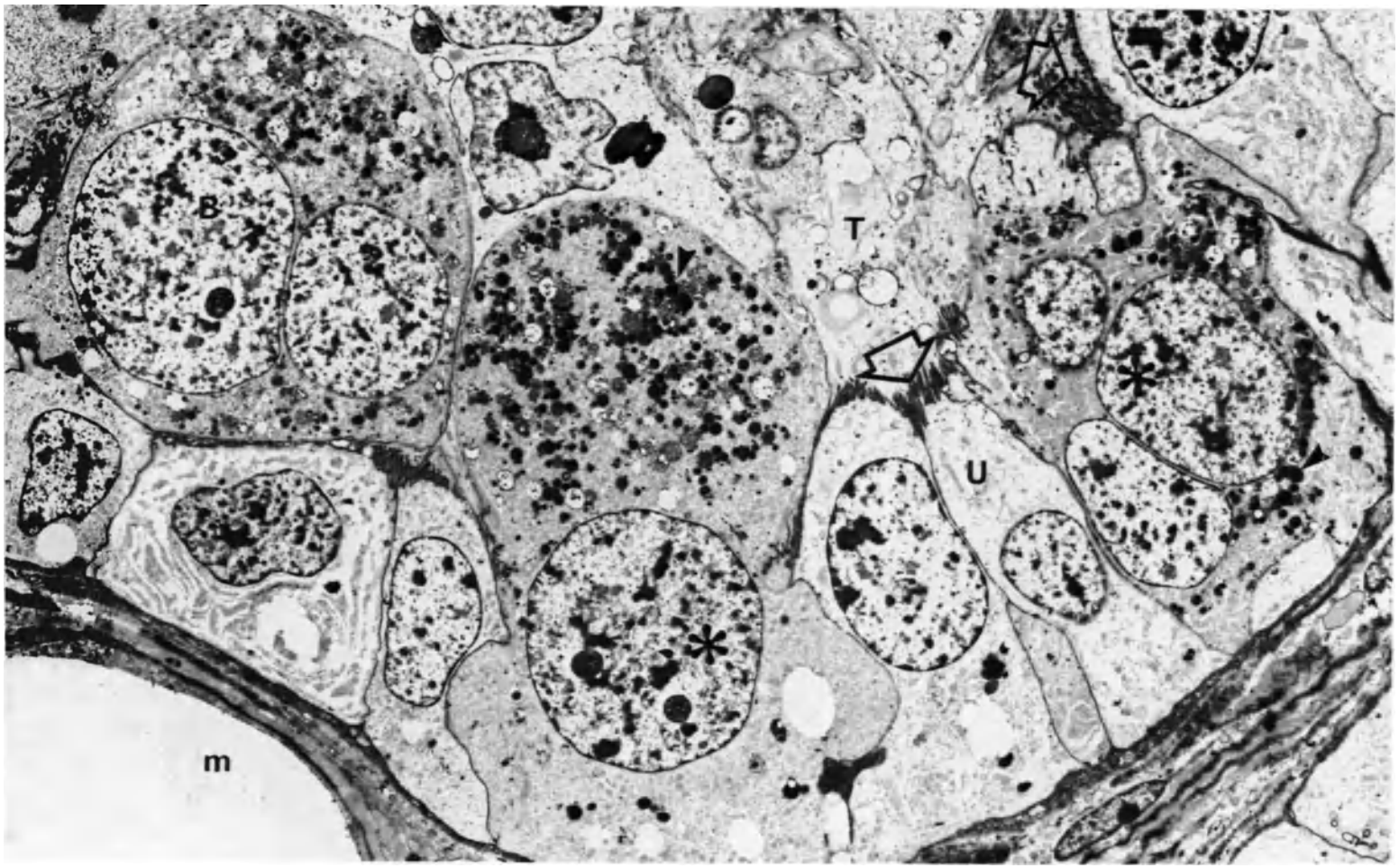


Figure 4.61 Synepitheliochorial cow placenta. For details see legend to Figure 4.60. 130 dpc, × 2700.

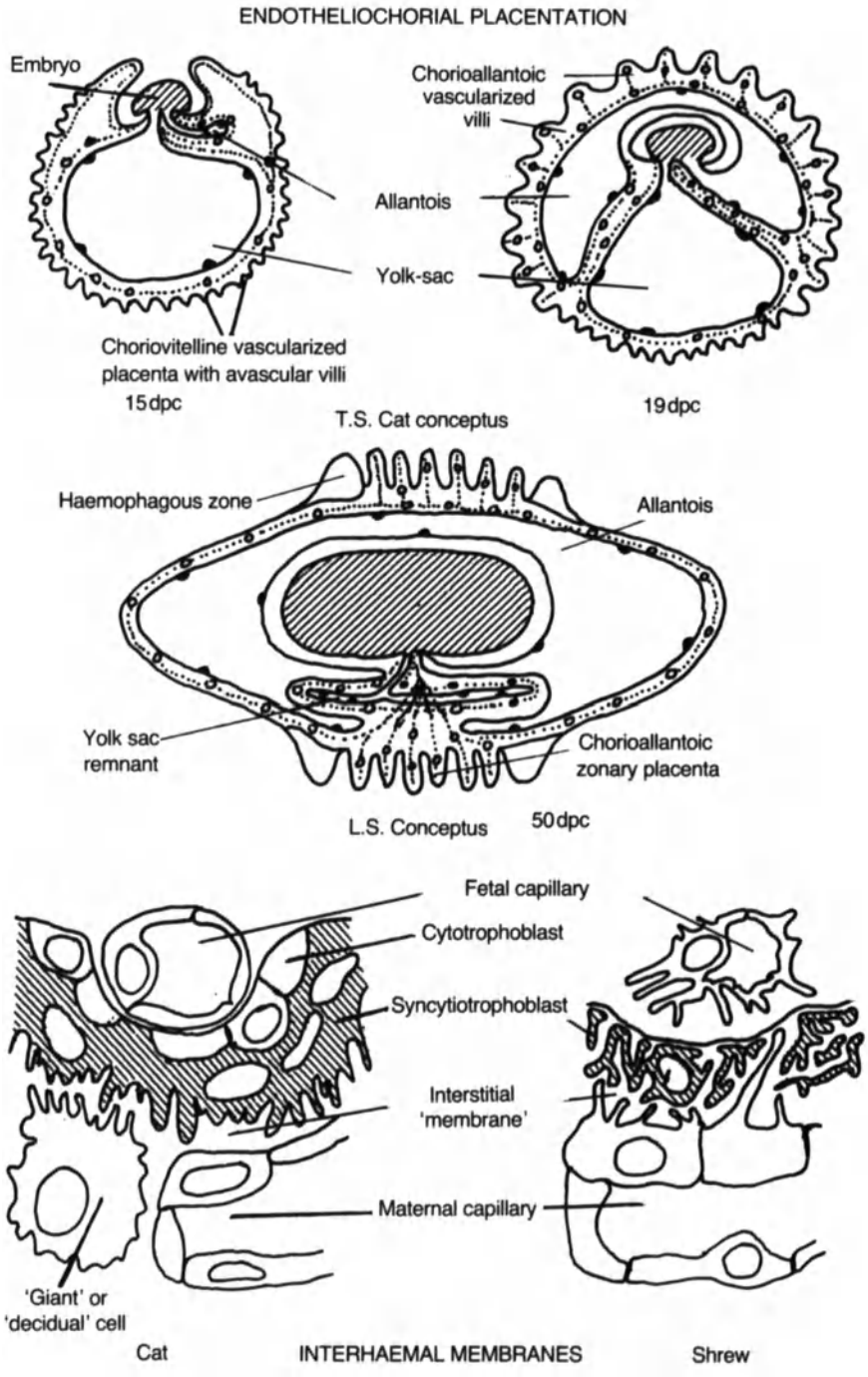


Figure 4.62 Placental membrane development in endotheliochorial placentation, mostly Carnivora.

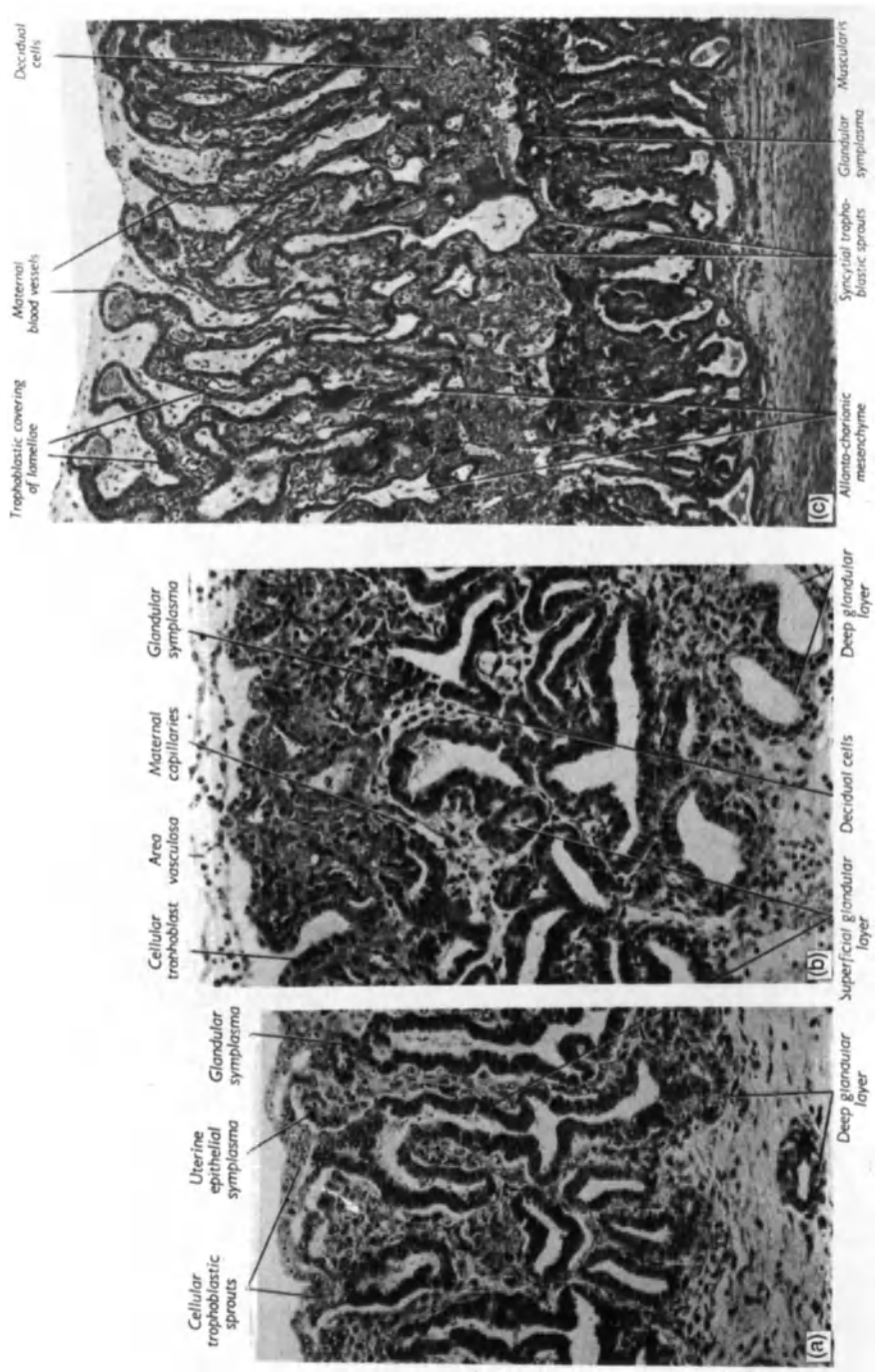


Figure 4.63 Endotheliochorial cat placenta (see Figure 4.62 for diagrams). Developmental sequence all at the same magnification, showing (a) early apposition (13½ dpc), (b) short yolk sac placental villi, non-vascularized (about 16 dpc) and (c) vascularized fetal chorioallantoic villi or lamellae (25 dpc). Note the increase in length of both the fetal villi and maternal crypt sides but the relative constancy of the depth of the glands plus junctional zone. (a) 13½ dpc, × 120. (b) 16 dpc, × 120. (c) 25 dpc, × 120.

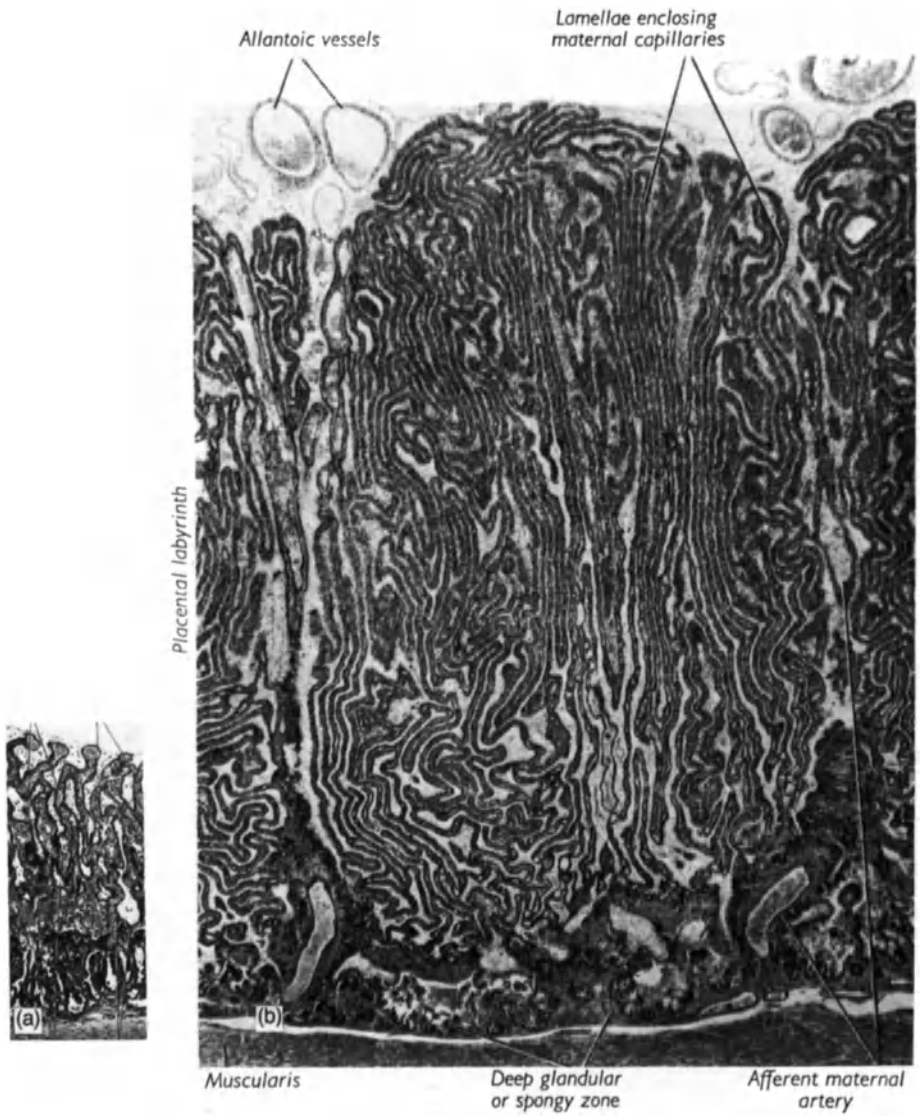


Figure 4.64 Endotheliochorial cat placenta. Placental development showing the enormous increase in length and proliferation of the labyrinthine fetal and maternal lamellae between 25 dpc (a) and about 60 dpc (b) (term is 61–63 dpc). Both (a) and (b) are at the same magnification. (a) 25 dpc, × 24. (b) 60 dpc, × 24.

fetus grows, thus continuously improving the transport potential of the placenta (Baur, 1977).

When the chorioallantoic membrane is first forming, the whole conceptus is expanding and elongating to become sausage shaped, with paraplacental regions outside the central zone of the placental villi. These regions have unchanged cellular trophectoderm and uterine epithelium loosely apposed with small areolae over the mouths of the numerous glands which are actively secreting throughout pregnancy (Leiser and Enders, 1980a). The area of this paraplacental region (Figures 4.62 and 4.65) is macroscopically larger than the zone of the placental villi but probably less than 5% of the total interhaemal area at term.

A characteristically large haemophagous zone develops at the edge or in the middle of the zonary endotheliochorial placenta of carnivores (Figure 4.65). It is lined on the fetal side by high columnar trophectoderm showing all stages of very active phagocytosis and digestion of the red blood cells from the stagnant pool of blood at the fetomaternal interface. On the maternal side the uterine epithelium may be either cellular or syncytial. Transient rupture of the occasional maternal blood vessel is probably instigated by the fetal trophectoderm at the edge of the haemophagous zone, but the details of the mechanism are unclear. The endothelial break is soon repaired and the uterine epithelium reforms over the top (Leiser and Enders, 1980b).

The gross form of the haemophagous zone or organ is very characteristic of the species. Cats and dogs (Figure 4.65) have green or brown borders to their zonary placentas coloured by blood pigment residues, and in the sea otter there is a large central pedunculate sac. There is an excellent review of haemophagous zones by Burton (1982).

The intimate relationship of the fetal and maternal blood vessels in the cat and the relative volumetric insignificance of the tissue in between is elegantly demonstrated by Leiser's SEM study of corrosion casts of the two vasculatures. These clearly show that maternal blood passes directly to the fetal side of the placenta and fetal blood to the maternal side prior to flowing back through their capillary systems. The arrangement of the arteriolar supply to the capillary networks

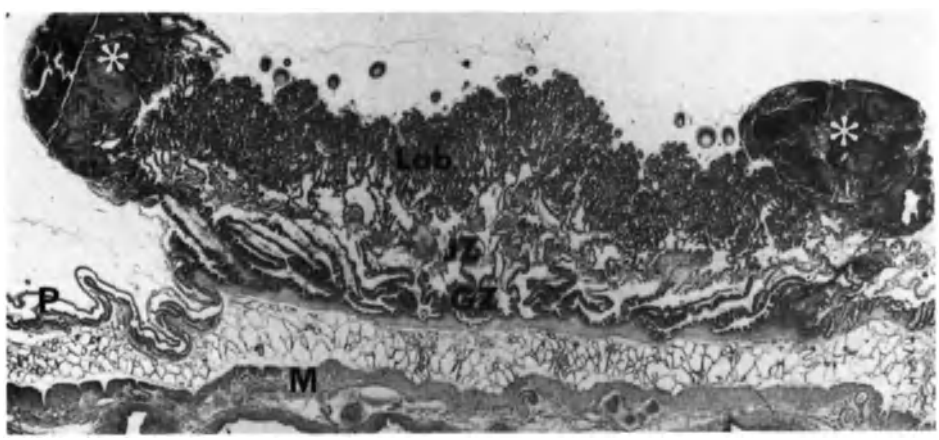


Figure 4.65 Endotheliochorial dog placenta. Cross-section through the zonary placenta of the dog at mid-pregnancy. Note the large marginal haemophagous zones (asterisks). Lab, labyrinthine chorioallantoic placenta; JZ, junctional zone; GZ, glandular zone; M, muscle in uterine wall; P, paraplacental chorioallantoic placenta. About 35 dpc, × 15. (From Bjorkman, 1970.)

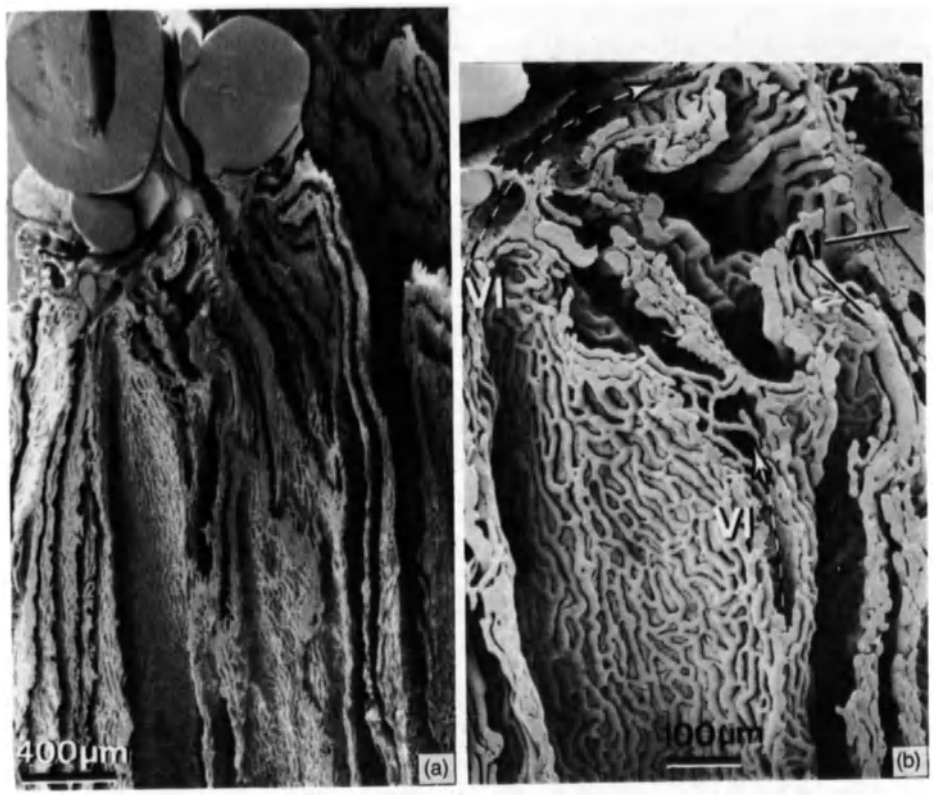


Figure 4.66 Endotheliochorial cat placenta. Corrosion casts of resin-injected vasculature. (a and b) fetal and, Figure 4.67, maternal blood vessels. Note the lamellae formed from the fetal capillary meshwork in the labyrinthine zone in (a) and (b). The casting resin filled fetal arterioles (AI) and capillaries but not the venules (VI), whose lumina are indicated by the dashed arrows in (b). (a) 57 dpc, × 25. (b) 57 dpc, × 100. (From Leiser and Kohler, 1984.)

is such that Leiser considers the flow to be predominantly cross-current (Figures 4.9, 4.66 and 4.67) (Leiser and Kohler, 1983).

Erosion of the original endometrial cells in the dog and cat is practically complete except for a population of cells which are distributed fairly evenly throughout the placenta in the amorphous (interstitial) material between fetal syncytium and maternal endothelium (Figure 4.68).

Wislocki and Dempsey (1946) suggested that these cells originate from fibroblasts. They are typically large, 30–50 μm with a large, or often two large, nuclei. They resemble fibroblasts in their massive fibrillar tracts but also have the swollen rough endoplasmic reticulum and large Golgi apparatus which suggests they may have considerable synthetic and secretory potential (J.W. Anderson, 1969; Malassine, 1974). Once the placenta is established these cells can divide and maintain their frequency as the placenta grows. They are very similar in ultrastructure and position to the residual endometrial cells described above for the sheep and goat synepitheliochorial placentas, and their function is equally enigmatic (Figure 4.69).

Descriptions of endotheliochorial placental development refer to a zone of necrosis in the endometrium at the tips of the advancing fetal villi. In this region the gland epithelium becomes syncytial, the connective tissue

oedematous, and both degenerate. From transverse sections of the placenta the impression given is that the necrotic zone represents an area of total tissue breakdown induced by the fetal syncytium, which then phagocytoses the resulting debris (Amoroso, 1952; Barrau *et al.*, 1975). The process is in fact very selective since the maternal blood vessels pass through this zone completely unchanged and they probably represent 30–50% of the volume of the structures initially present (Leiser and Kohler, 1983). The closest analogy might be a building on extensible stilts, which are sufficiently robust to support and supply the building of the upper floors. These would correspond to the initial chorio-vitelline placenta. The blood vessel 'stilts' would eventually be incorporated into the final structure by growth of the chorioallantoic lamellar placenta. The initial distribution of the maternal vasculature would thus determine the basic pattern of placental development. To accurately test this hypothesis would require corrosion cast studies during placental development, since both volume and distribution of the blood vessels would be critical to the process.

The characteristics of carnivore placentation are remarkably consistent. The initially weakly vascularized endotheliodichorial villous choriovitelline placenta is colonized by the rapidly proliferating allantoic vasculature

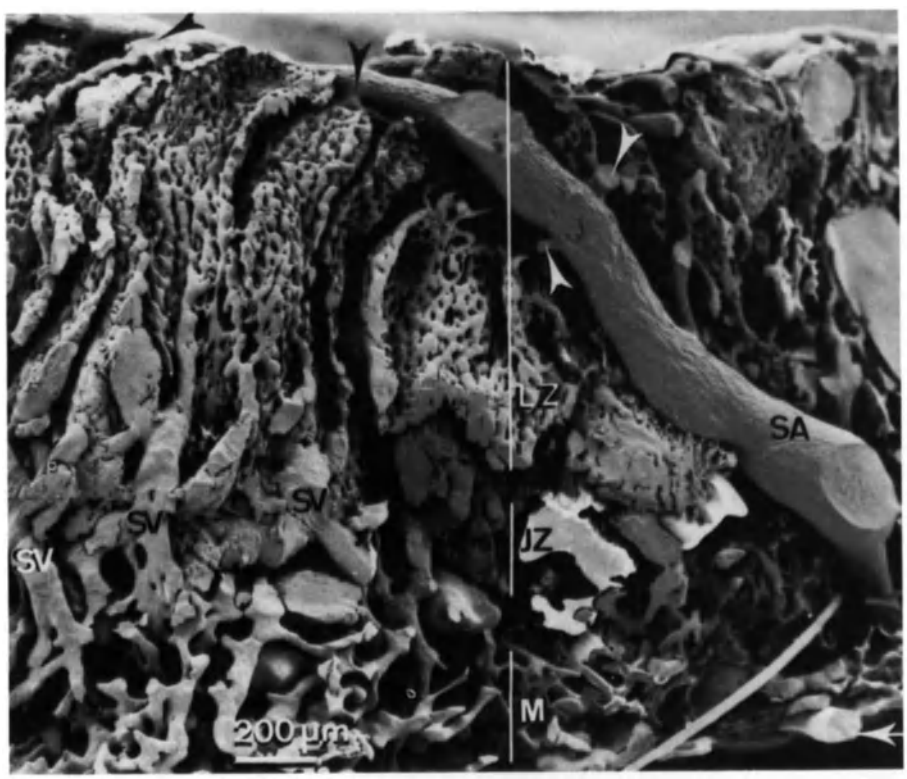


Figure 4.67 Endotheliochorial cat placenta. Corrosion cast of maternal blood vessels. The maternal stem artery (SA) runs through the full depth of the placenta before supplying (arrowheads) the capillaries of the labyrinthine zone (LZ). The capillaries collect into broad venules (SV) in the junctional zone (JZ), which drain into veins (white arrow) in the endometrial muscle layer (M). Compare this figure with the histological cross-section of the dog placenta in Figure 4.65. 22 dpc, × 12. (From Leiser and Kohler, 1983.)

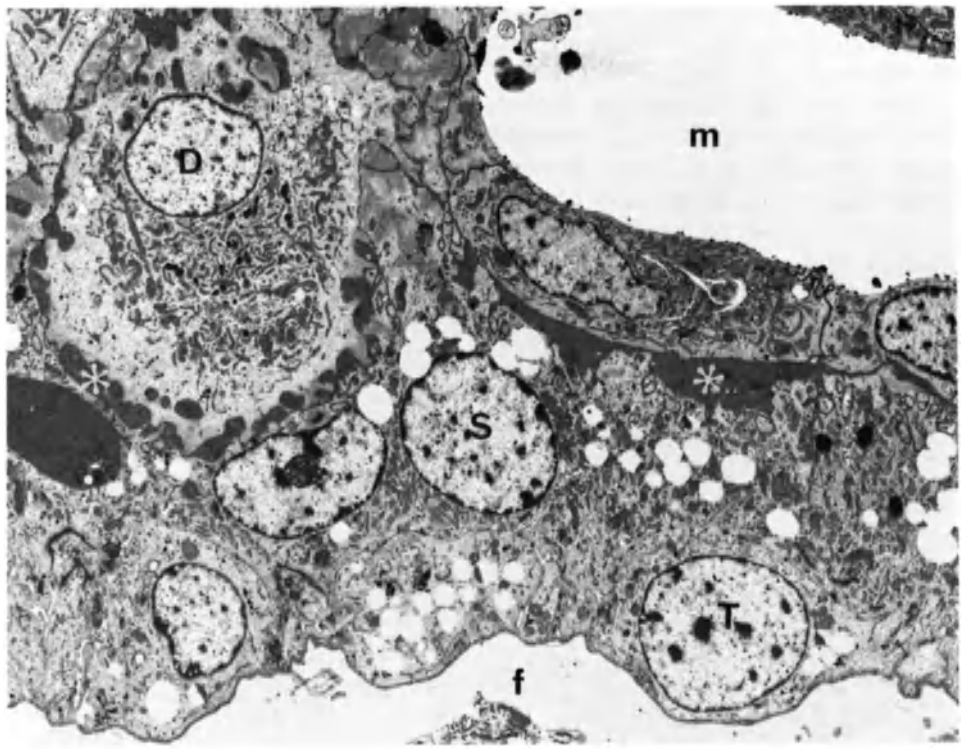


Figure 4.68 Endotheliochorial placentation. Electron micrograph of the definitive interhaemal layers in a cat placenta. 'Decidual' cells (D) in the maternal connective tissue are consistently present. They have extensive rough endoplasmic reticulum, large Golgi bodies and glycogen deposits. This ultrastructure is similar to that seen in cells in a similar position in sheep placenta (see Figure 4.69). f, fetal connective tissue; T, cellular trophectoderm; S, syncytial trophectoderm; asterisks, 'interstitial layer'; m, maternal blood vessel. 35 dpc, × 3000.

to produce the endotheliodichorial chorioallantoic placenta. This is always associated with a haemophagous zone, a large allantoic sac and a significant yolk sac remnant. The only exception so far reported is the hyena, which has the typical large allantois and persistent yolk sac but a haemomonochorial definitive placenta (Figure 4.10a) which still has a marginal sinus similar in size to that of the cat haemophagous zone but filled with circulating rather than static maternal blood (Dempsey, 1969; Oduor-Okelo and Neaves, 1982; Wynn *et al.*, 1990).

There are even more intermediate stages between endotheliochorial and haemochorial structure shown by bats (Chiroptera) (Bhiwgade, 1990; Rasweiler, 1993), of which the families with the more primitive characteristics are endotheliochorial with clearly defined (Emballonuridae) or more diffuse (Megadermatidae) equivalents of a haemophagous zone (Gopalakrishna and Karim, 1979; Mossman, 1987). There is far more destruction of the maternal endometrium by the bat trophectoderm in the haemophagous zone than in carnivores and plainly, if the erosion continued to encroach on the main placenta, the haemochorial type would result, as seen in Desmodontidae (Bjorkman and Wimsatt, 1968), Molossidae (Stephens, 1969; Stephens and Cabral, 1971, 1972; Rasweiler, 1990, 1991), Phyllostomatidae

(Bodley, 1974; Bleier, 1975), Thyropteridae (Wimsatt and Enders, 1980) and Vespertilionidae (Kimura and Uchida, 1984), Pteropodidae and Hipposideridae (Bhiwgade, 1990). Rasweiler (1991) has recently provided an excellent account of the development of the haemomonochorial placenta in *Molossus ater*.

It is possible that the intrasyncytial lamella (ISL) present in all haemochorial bats so far examined closely (Figures 4.11 and 4.99) may be analogous to the endotheliochorial interstitial membrane (Rasweiler, 1993). The ISL consists of a discontinuous system of membrane-bound channels filled with basement membrane-like material. It is usually sporadically continuous with the maternal blood space, but in one molossid bat (Stephens, 1969) is continuous with the fetal syncytiotrophoblast basement membrane instead (Figure 4.11). Transport and skeletal functions have been suggested (see section 4.2.7a). Other families of bats (Hipposideridae, Noctilionidae, Rhinolophidae and Rhinopomatidae, (J.W. Anderson and Wimsatt, 1963; Gopalakrishna and Karim, 1979; Mossman, 1987) are said to have endotheliochorial placentas but no haemophagous zone – thus that linkage is by no means absolute. The endothelial nature of the definitive placenta of the Rhinopomatidae, Emballonuridae, Megadermatidae and

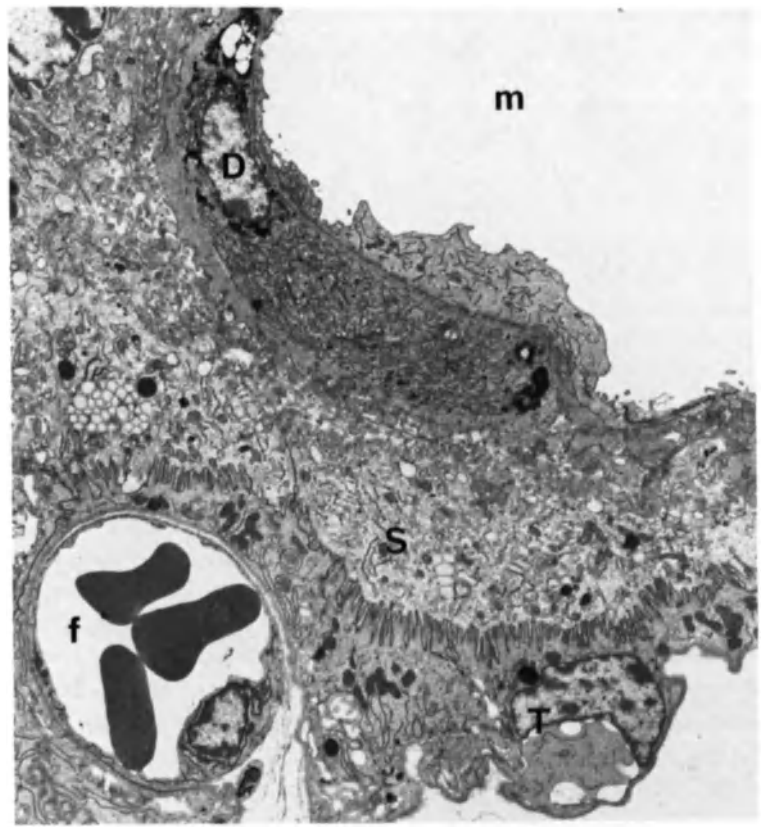


Figure 4.69 Synepitheliochorial placentation. The (maternal) endometrial connective tissue in the mature sheep placenta has 'decidual cells' (D) with similar ultrastructure and in a similar position to those found in cat placenta (Figure 4.68). f, fetal blood vessel; T, cellular trophectoderm; S, syncytial fetomaternal trophectoderm; m, maternal blood vessel. 140 dpc, × 4000.

Rhinolophidae has now been clearly established in a recent comparative electron microscope study (Bhiwgade, 1990). This supports the light microscope evidence that the Noctilionidae are also endotheliochorial (J.W. Anderson and Wimsatt, 1963).

It was only by a study of the ultrastructure that the placenta of the insectivore *Blarina* (common shrew) was shown to be endotheliochorial (Wimsatt *et al.*, 1973). The syncytial trophoblastic layer is uniquely attenuated and profusely fenestrated, and both maternal and fetal capillary endothelial cells extend slender processes from their bases to or through this lace-like trophectoderm (Figure 4.70b).

It certainly would be no barrier to macromolecules but may have some residual role in regulation or prevention of maternofetal cell traffic. A recent suggestion that in the final week of pregnancy it forms no significant barrier at all (Kiso *et al.*, 1990) requires confirmation.

The shrews (Soricinae) also have an unusual annular haemophagous zone with absorptive trophectoderm underlain by vitelline (yolk sac) capillaries (B.F. King *et al.*, 1978) rather than the usual allantoic vascular system as in carnivores or bats. This emphasizes again the plasticity of the fetal membranes with quite different parts adapting to serve the same function. Fenestrated syncytiotrophoblast has been found in only one other order, Bradypodidae, the sloths, again one with an endotheliochorial placenta. Here the layer is more substantial than in the shrews but still essentially porous. There is no haemophagous zone and the rest of the fetal membranes are, according to Mossman (1987), more reminiscent of anthropoids with a very early vascularization of the eventually discoid chorioallantoic placenta and the small non-persistent yolk sac (B.F. King *et al.*, 1982).

Other examples of endotheliochorial placentation very similar to the carnivore interhaemal membrane in structure are found in widely separated orders. Some of these have an accompanying haemophagous zone, as in the Elephantidae (Perry, 1974), but most [the aardvark (Orycteropodidae), two insectivores, *Talpa* (Malassine and Leiser, 1984) and *Tupaia* (Luckhardt *et al.*, 1985), and the rodent, *Dipodomys* (Figure 4.71) (the kangaroo rat, B.F. King and Tibbitts, 1969)] have no equivalent structure.

The endotheliochorial placenta thus appears to be a successful solution for a variety of animals in a wide range of habitats with very different reproductive habits and maternal sizes. A haemophagous zone is a sufficiently frequent accompaniment to endotheliochorial placentation to indicate one advantage in developing a fully haemochorial placenta. Most orders with endotheliochorial placentas also have members with the haemochorial type.

(d) Haemochorial placentation (mouse, rabbit, man, apes, monkeys)

The epitheliochorial–synepitheliochorial–endotheliochorial sequence represents a gradual loss of interhaemal layers. The haemochorial is an apposite end to such a sequence; with the loss of all the maternal tissue, maternal blood irrigates the chorioallantoic fetal trophectoderm directly (Figure 4.5).

Previous suggestions that in some species the fetal trophectoderm is also lost have not been substantiated by subsequent EM studies. The trophectoderm may be very thin but no placenta yet accurately described lacks a trophectoderm or its derivative as a barrier for the maternal blood, although pores in the trophectoderm have been reported (B.F. King, 1992). Depending on the species the trophectoderm may have one, two, or three layers, each of which may be cellular or syncytial. The species-specific pattern is established soon after implantation and is maintained to term.

Fetal placental tissue is usually aggregated in a discrete area, discoid or limited zonary.

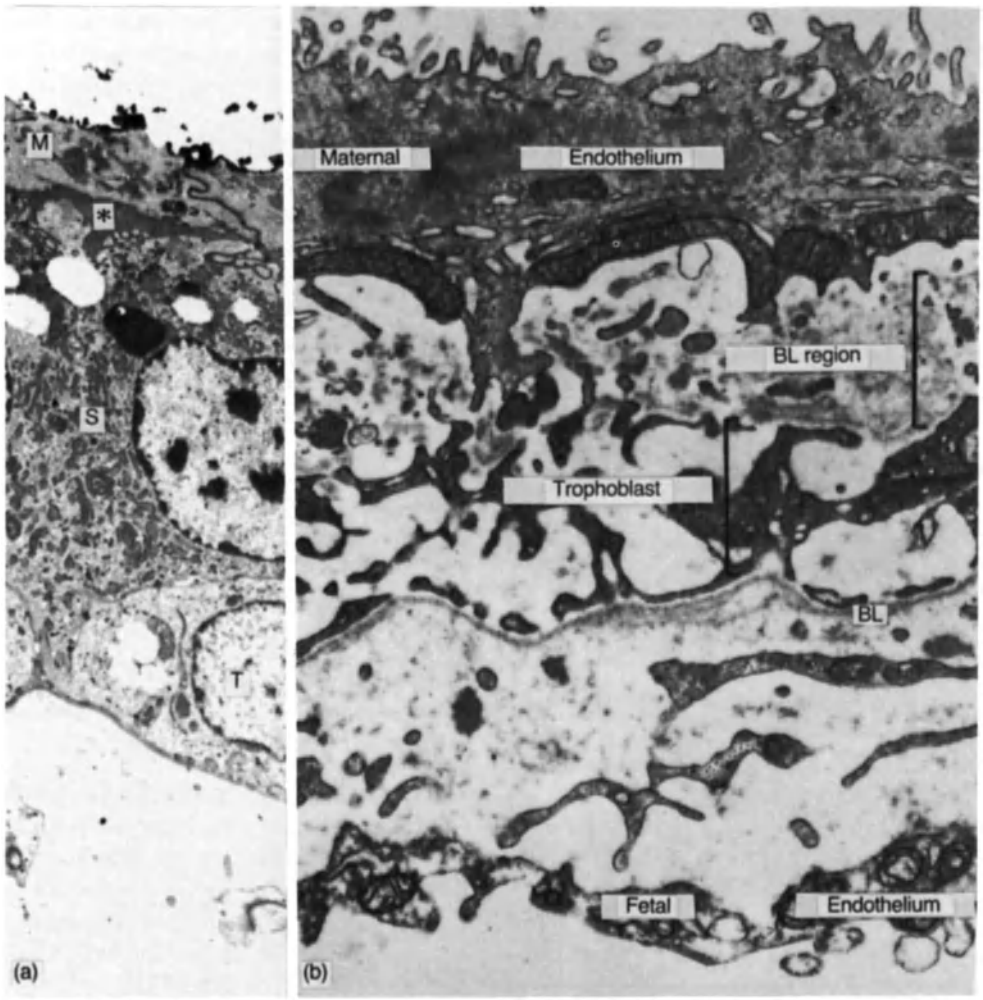


Figure 4.70 Most endotheliochorial placentas display a fairly uniform interhaemal membrane (see Figure 4.68), as typified by the cat (a) with a discontinuous cellular cytotrophoblast (T) which generates the thick syncytial layer (S) separated from the maternal endothelium (M) by a dense acellular interstitial layer (asterisk). However, the insectivore, *Blarina* (b) with its exiguous discontinuous meshwork of syncytiotrophoblast, and the rodent, *Dipodomys* (Figure 4.71), with its extremely thin syncytiotrophoblast layer (Figure 4.71, arrows) clearly demonstrate the variability possible within the endotheliochorial framework. The interstitial layer (asterisk in a; 'BL region' in b; arrowheads in Figure 4.71) is also very different in these examples. BL, trophoblast basement membrane; Fc, fetal capillary; M, maternal capillary. (a) 35 dpc, × 8000. (b) Near-term placenta, × 18 100. (From Wimsatt *et al.*, 1973.)

The structure of the paraplacental area varies, with the yolk sac placenta playing an important role throughout pregnancy in rodents, lagomorphs and insectivores (Figures 4.2 and 4.3) but insignificant in primates. No areolar development has been reported; the glands do not play any significant role except very early in pregnancy.

The erosion of the maternal tissue is considerable, and typically the species with hae-

mochorial placentation induce decidual tissue transformation in the endometrium during placental formation. Again the variation is enormous, with massive decidua in the rodent, moderate in the human and negligible in the rhesus monkey.

It must be emphasized that, although most maternal tissue is lost, major maternal arterial channels persist throughout pregnancy through the full depth of the placenta in most haemochorial placentas supplying maternal blood to the fetal surface (subchorial lakes) of the placenta. Usually several channels persist (rabbit, see below), sometimes only one [in the insectivorean elephant shrew (Starck, 1949)] (Figure 4.72).

The most notable exceptions to this are man, apes and the Old World monkeys.

Haemochorial placentas are rather more varied in gross architecture than previous types with villous or labyrinthine structure and a variable and specific number of interhaemal layers.

As examples of the labyrinthine type with haemotrichorial structure the rat and mouse will be discussed in detail with rabbit (haemodichorial) and man, (haemomonochorial) for comparison.

Haemotrichorial: mouse, *Mus musculus;* rat, *Rattus rattus*

Oestrous cycle: 4–7 days
Ovulation: spontaneous
Uterus: duplex
Litter: 5–9
Gestation: 19 days (mouse) 22 days (rat)
Implantation: interstitial
Amniogenesis: cavitation
Yolk sac: Initial bilaminar yolk sac stretched to disappearing point by mid-pregnancy. No choriovitelline placenta formed. Completely inverted well-vascularized splanchnopleuric yolk sac placenta with villous mesometrial half persists until term (Figure 4.2).
Chorioallantois: Forms definitive placenta, vascularized by allantoic mesenchymal bud (Figures 4.2 and 4.75). The allantoic sac is vestigial or absent.
Shape: discoid, labyrinthine structure
Decidua: extensive development throughout

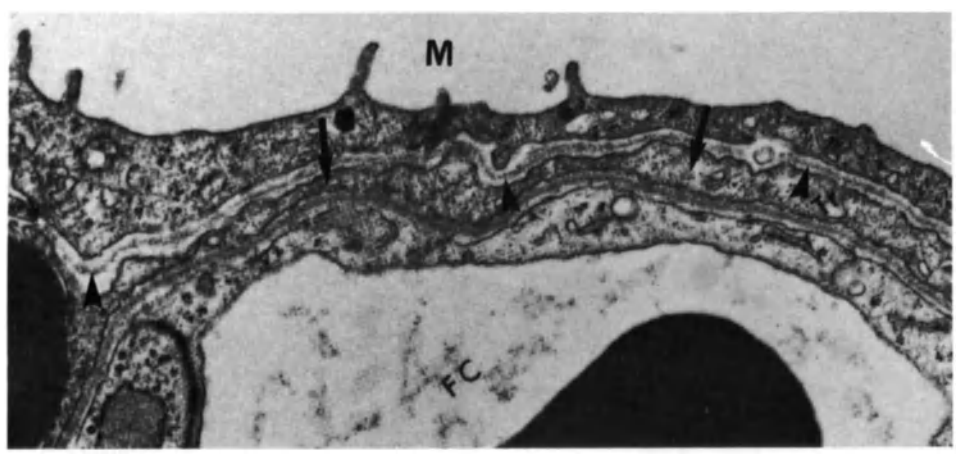


Figure 4.71 Endotheliochorial placentation. The rodent *Dipodomys* has very thin but continuous syncytiotrophoblast (arrows) and interstitial (arrowheads) layers. Compare these structures with those in cat (Figure 4.70a) and shrew (Figure 4.70b) placentas. FC, fetal capillary; M, maternal capillary. Near-term placenta, × 18 000. (From King and Tibbits, 1969.)

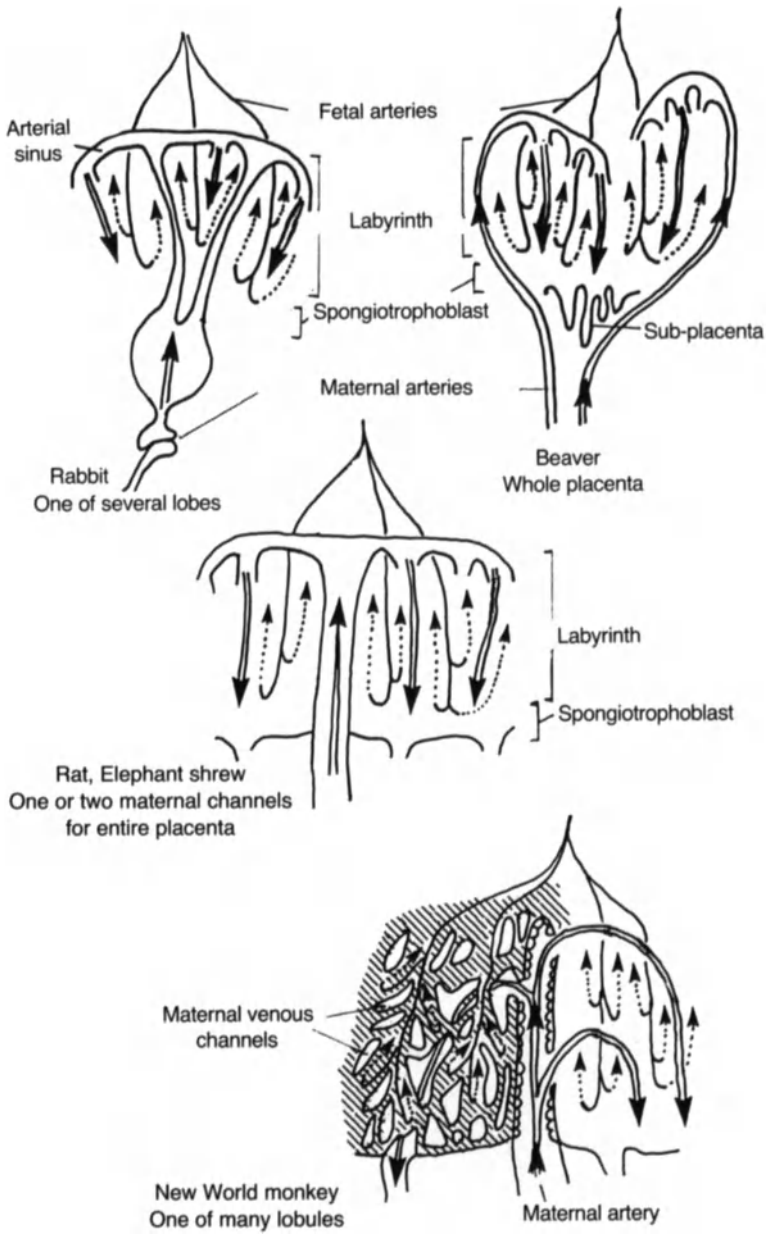


Figure 4.72 Schematic circulations in haemochorial placentas. See also Figures 4.8 and 4.9.

pregnancy in the endometrium around the conceptus (see Figure 4.12)

Interhaemal membrane: haemotrichorial labyrinthine, two syncytia next to the fetal tissue and a cellular layer lining the maternal blood space

Accessory placental structure: completely inverted yolk sac

Fetal membranes and placental development

In these rodents the blastocysts reach the uterine lumen on 3–4 dpc and implant after

uterine contractions, finally influenced by the individual blastocysts, have ensured an even spacing. Closure of the uterine lumen then immobilizes the blastocysts and ensures an intimate apposition between blastocyst and uterine epithelium (Enders *et al.*, 1980), a process which may involve specific changes in glycocalyx carbohydrate expression (Kimber and Lindenberg, 1990; Kramer *et al.*, 1990). There is no appreciable swelling of the blastocyst prior to closure, and the blastocyst invariably implants at the antimesometrial side of the uterine lumen, with the embryonic disc mesometrial (Amoroso, 1952; M.H. Kaufman, 1983).

Implantation starts at 5 dpc with intrusion between, or possibly trophectodermal fusion with, the uterine epithelial cells (Figure 4.73) (Bevilacqua and Abrahamsohn, 1989) followed by complete death and delamination of the uterine epithelium (Enders *et al.* 1980; Schlafke *et al.*, 1985; Blankenship *et al.*, 1990 (hamster); Welsh and Enders, 1991a; Blankenship and Given, 1992).

At this stage the blastocyst is bilaminar but mesoderm is starting to form at the edge of the embryonic rudiment underneath the aggregation of trophectodermal cells referred to as the ectoplacental cone. The peripheral trophectoderm of the cone will differentiate into the polar or secondary giant cells which invade the uterine endometrium, by now considerably decidualized (M.B. Parr *et al.*, 1986) (Figure 4.12 and see section 4.2.8 above). Over the antimesometrial two-thirds of the blastocyst, away from the ectoplacental cone, the endodermal cells secrete material which thickens the basement membrane separating the endoderm from the mural trophectoderm which has differentiated into the primary giant cells (Fatemi, 1987; Mazariegos *et al.*, 1987). This thickened basement membrane is then referred to as Reichert's membrane (Figure 4.3). The mural or primary giant cells rupture the maternal blood vessels and form lace-like extensions through which the blood circulates but does not penetrate past Reichert's membrane (M.H. Kaufman, 1983; Welsh and Enders, 1987). The endoderm and primary giant cells then form a non-vascularized bilaminar yolk sac placenta. As the blastocyst develops and increases in size this bilaminar region maintains a similar structure but becomes ever thinner, eroding the decidua capsularis between the giant cells and the myometrium but finally completely rupturing on 17–18 dpc. This exposes the vascularized endodermal layer (referred to as the inverted visceral yolk sac placenta) to the newly restored uterine epithelium (Welsh and Enders, 1983) (Figures 4.3 and 4.12). Absorption of maternal immunoglobulins across this placenta throughout pregnancy is of considerable importance to neonatal immunoregulation (Bainter, 1986).

After the initial differentiation of the giant cells the yolk sac inverts beneath the embryo; above, the allantoic outgrowth from the fetal gut reaches the base of the ectoplacental cone (Figure 4.2). This has been beautifully demonstrated by SEM (Figures 4.74 and 4.75) (Ellington, 1985, 1987).

The cone appears to have three layers: inner, outer and a shell of secondary giant cells invading the mesometrial decidua basalis. As soon as the allantoic outgrowth reaches the bottom of the ectoplacental cone it erodes the endoderm and spreads out into a mesodermal pad closely underlying the inner ectodermal layer of the cone. An excellent pictorial sequence of placental membrane and embryonic development can be found in Rugh (1975).

Considerable structural reorganization together with the stimulation of rapid cellular division in the cone tissue follows and outgrowths of cone cells into the decidualized endometrium initiate the discoid chorioallantoic placenta (Welsh and Enders, 1991b; Enders and Welsh, 1993) (Figure 4.76).

By this time the outer giant cell layer is irrigated by maternal blood and is actively eroding the maternal decidual tissue and

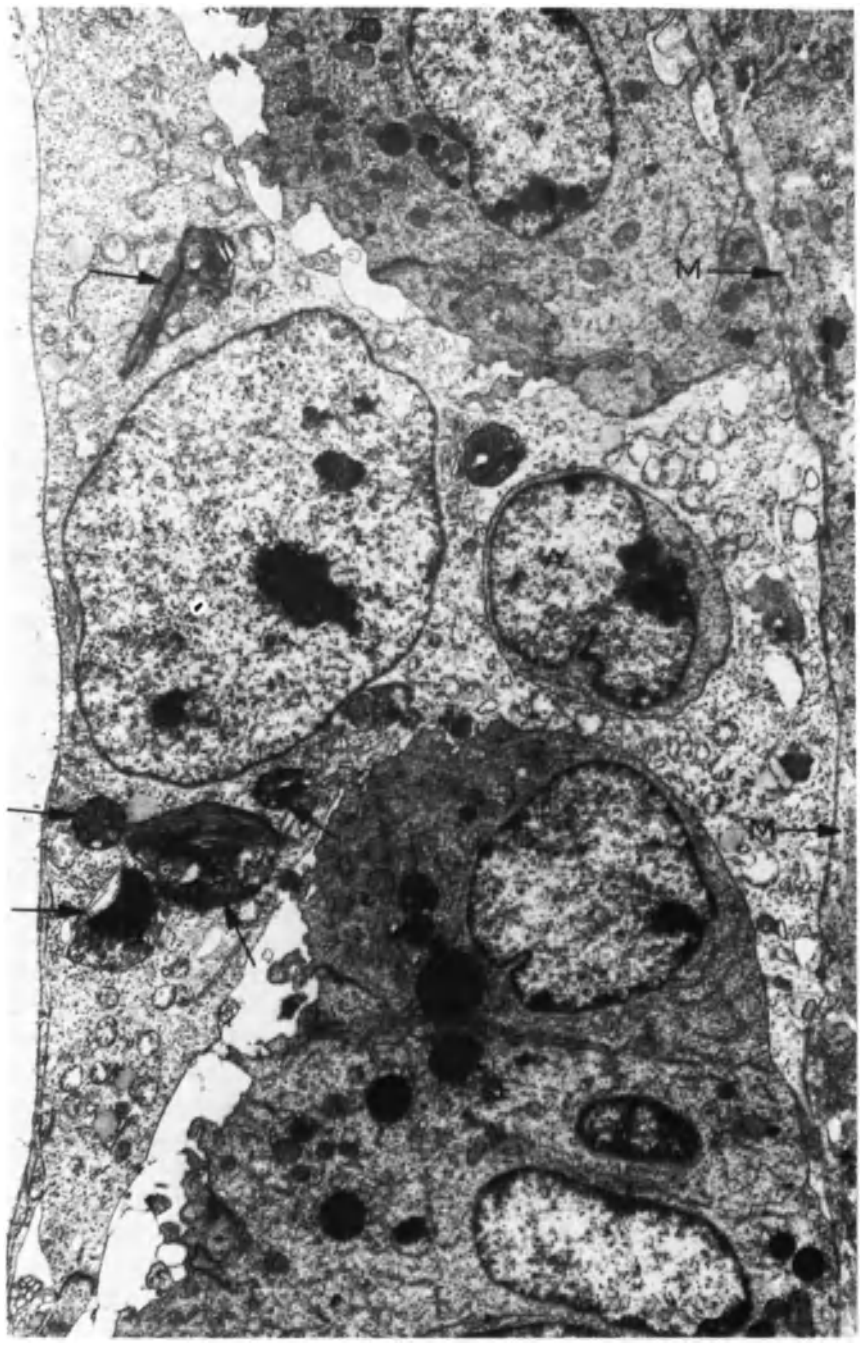


Figure 4.73 Haemochorial placentation. Early implantation in the mouse. A trophectoderm cell (T) containing several lysosomes (arrows) has completely penetrated through to the basement membrane of the uterine epithelium (M), apparently phagocytosing or flowing round a uterine epithelial cell (A) in the process. This could be the result of intrusion or fusion; adjacent uterine epithelial cells seem unaffected. 5½ dpc, × 3450. (From Kaufman, 1983.)

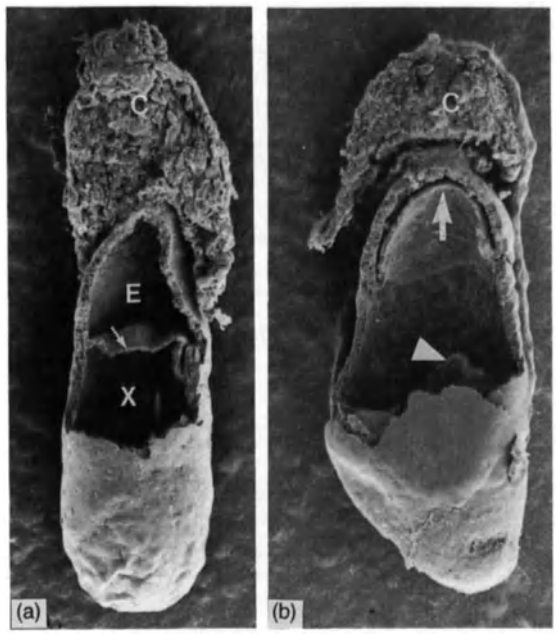


Figure 4.74 Scanning electron micrographs of the rat conceptus after removal of the parietal yolk sac, all at the same magnification, dissected to show the internal membrane development. (a and b) Expansion of the exocoelom (X) inverts the chorion (small arrow) which bounds the ectoplacental cavity to form a double layer of trophectoderm lined with mesoderm (large arrow) under the ectoplacental cone (Figure 4.75a). This forms the basis for development of the allantochorionic placenta when vascularized by growth (Figure 4.75a and b) of the solid mesodermal allantois (arrowheads) which originates from the hind gut of the embryo, here covered by the amnion (A). (a) 9.25 dpc, × 220. (b) 9.25 dpc, × 220. (From Ellington, 1985, 1987.)

expanding into the vascular sinuses. Giant cells do not divide, but are replenished by smaller cells at the surface of the ectoplacental cone. The allantoic vasculature is aggressively growing into the tissues at the base of the ectoplacental cone. The cone is considerably differentiated and is starting to form villi [Metz, 1980 (rat); Carpenter, 1972, 1975 (hamster, which is also haemotrichorial)] (see Figure 4.76). As these villi grow they too extend into the maternal blood sinuses. The fetal blood is then separated from the maternal by three layers, two syncytia derived from the inner ectoplacental cone layers and the outer cellular layer grading into the giant cells. These are the basic interhaemal layers through the rest of gestation and the usual nomenclature is maternal blood/layer I/ layer II/layer III/fetal endothelial basement membrane/fetal endothelium/fetal blood (Figure 4.76).

The fetal villi become confluent with one another as they increase in length at the expense of the decidua basalis tissue, eroding the maternal venous capillaries and colonizing the sinuses. The main maternal arterial channels are not destroyed (about 4–7 per discoid placenta) but run through the whole thickness of the placenta directly to the subchorial lake at the base of the fetal placenta, from where the maternal blood flows back through the labyrinthine network formed by the fetal tissue growth (Figures 4.77 and 4.78).

The persistent maternal arterial channels are initially lined with maternal endothelium but this is displaced and phagocytosed by giant cells at an early stage. The process eventually produces widened channels lined with fetal cells (endovascular trophectoderm) extending as far as the uterine muscle layer [Legrand, 1974 (rat); Pijnenborg *et al.*, 1981 (man); Carpenter, 1982 (hamster); Hees *et al.*, 1987 (guinea pig)].

Growth of a haemotrichorial placenta like that of the rat or mouse presumably is based upon small groups of mitotically active cells continuous with the main attenuated layers (I, II and III) which separate fetal and maternal blood. Layers II and III are in fact syncytial from the earliest stage and, as no nuclear division has yet been reported in such syncytia, some system for growth and extension of the layers is necessary (Davies and Glasser, 1968; Metz, 1980). Layers II and III are very closely apposed and connected by numerous gap junctions whose function is obscure (Metz *et al.*, 1976) (see section 4.2.7a

above). The combined layers form a complete barrier to tracers introduced into maternal (Metz *et al.*, 1978) or fetal (Aoki *et al.*, 1978) circulations. The outermost layer (I) is cellular and fenestrated, and forms no barrier to tracers added to the maternal circulation. Enders (1965) has suggested that this layer I functions by slowing the maternal blood flow and forming local regions of blood stasis behind the fenestrations which facilitate feto-maternal transport. There may be special difficulties in fetal uptake from maternal blood which are alleviated by such a slowing of the flow. Clearly a balance is needed between rapid flow to maintain solute concentrations and efficient capture of the substrates by the translocases in the plasmalemma. All three layers (I, II, III) attenuate during pregnancy but no significant ultrastructural changes have been reported (Jollie, 1969, 1979; B.F. King and Hastings, 1977; Jollie and Craig, 1979; Metz, 1980). The first cells to differentiate are the primary giant cells around the antimesometrial and lateral aspects of the conceptus. At 6–7 dpc they form a more or less continuous shell around the conceptus with the secondary giant cells which differentiate subsequently around the

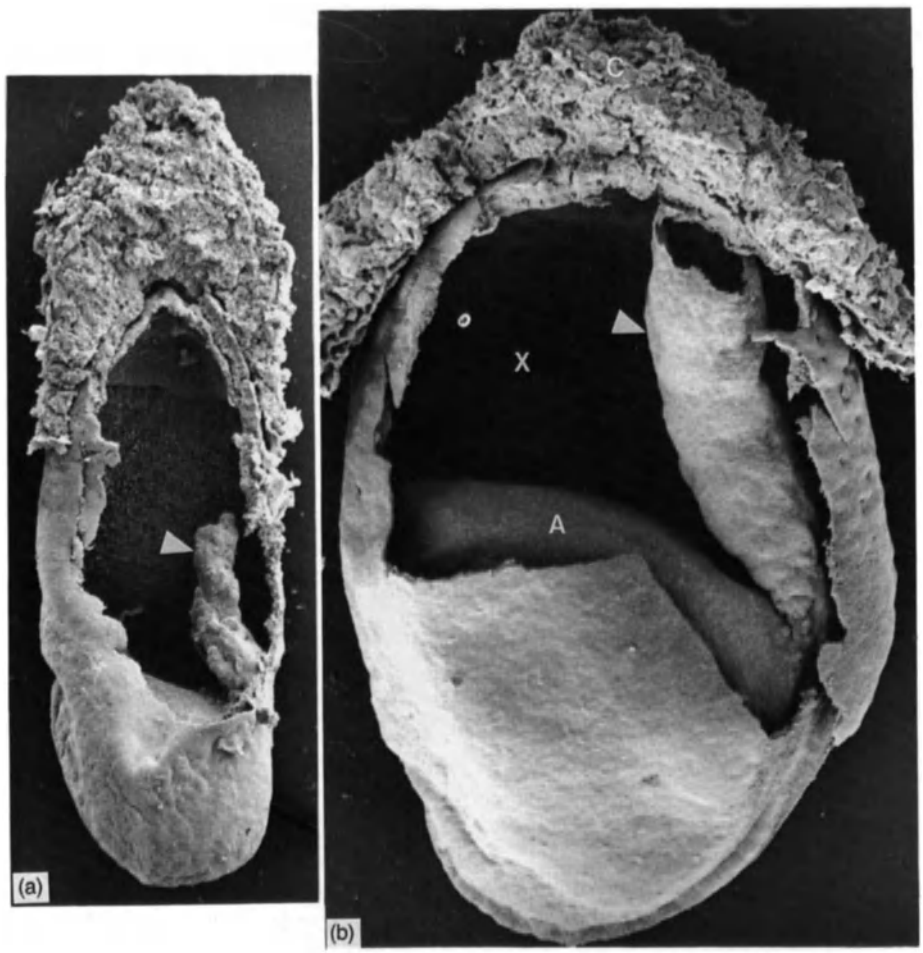


Figure 4.75 Scanning electron micrographs of rat conceptus. See Figure 4.74 legend for details. (a) 9.75 dpc, × 220. (b) 10.25 dpc, × 220. (From Ellington 1985, 1987.)

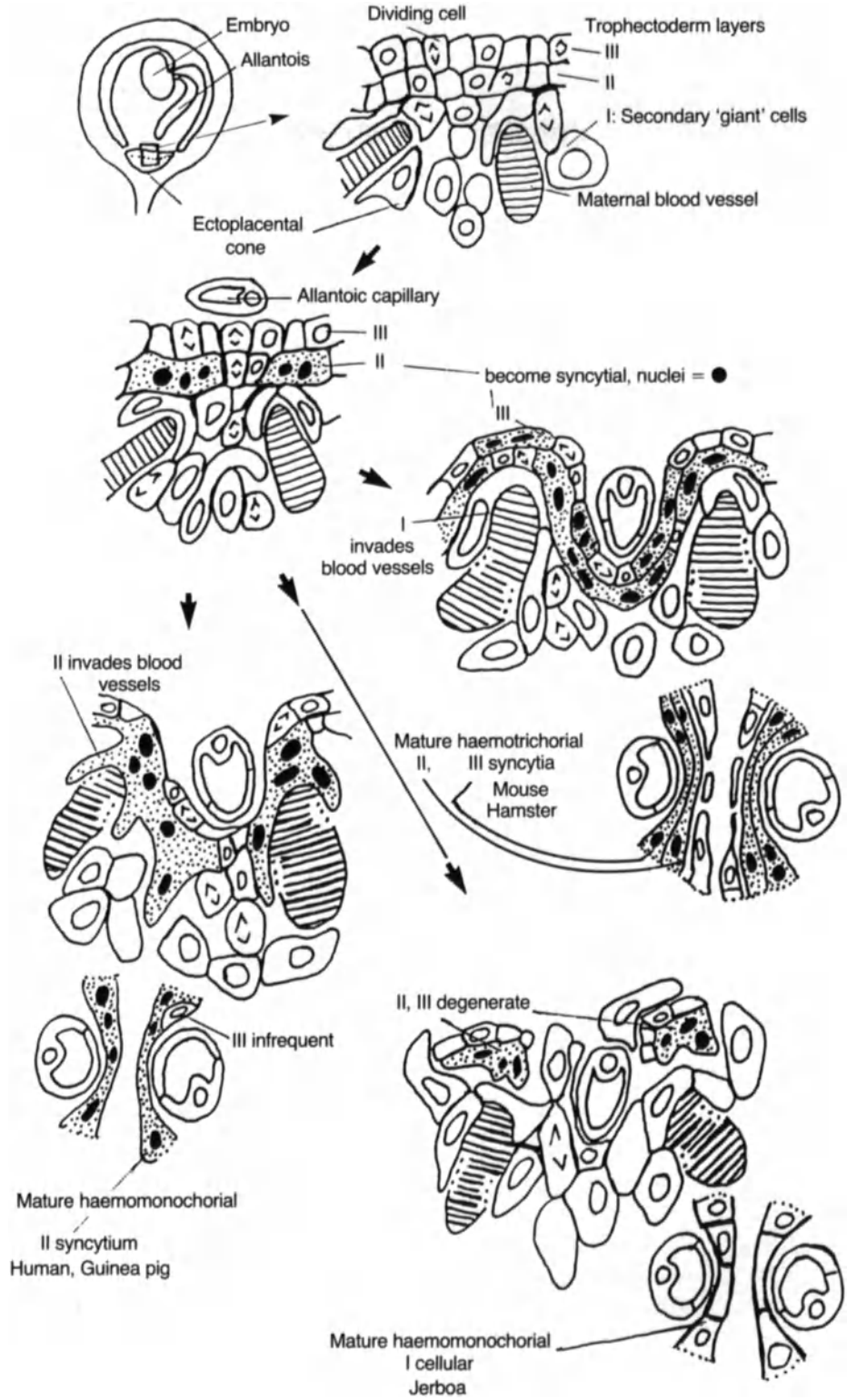


Figure 4.76 Placental development in multilayered haemochorial placentas.

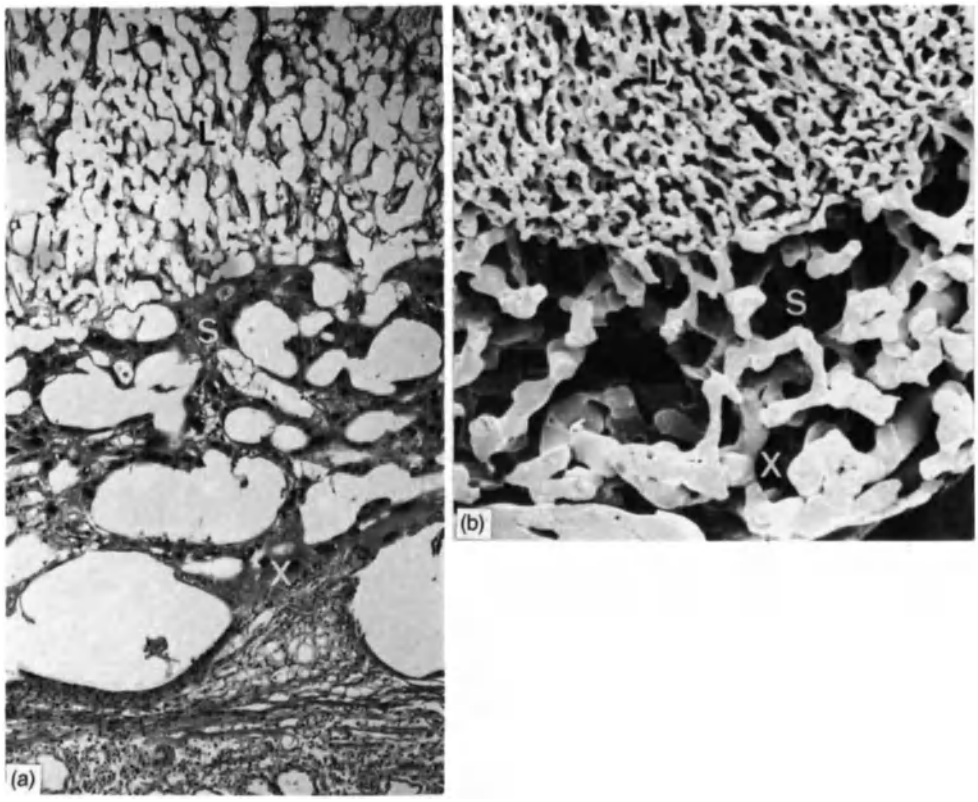


Figure 4.77 Haemochorial rat placenta, vascular patterns. (a) Section through and (b) equivalent corrosion cast preparation of the maternal vasculature in the near term rat placenta. Note the uniformly fine dimension of the maternal channels in the labyrinth (L) and the abrupt transition to the spongiotrophoblast (S) and giant cell (X) zones. (a) 22 dpc, × 66. (b) 22 dpc, × 50. (From Christofferson and Nillson, 1988.)

edge of the ectoplacental cone. The giant cells have been shown to produce steroids (Deane *et al.*, 1962), placental lactogens (rat, Campbell *et al.*, 1989; Duckworth *et al.*, 1990; mouse, Nieder and Jenne, 1990; hamster, Renegar *et al.*, 1990; reviews, Soares *et al.*, 1991; Southard and Talamantes, 1991) and relaxin (golden hamster, Johns and Renegar, 1990). The giant cells become very large with a characteristically enormous, deeply divided nucleus, of variable but usually very high DNA content (up to 1024 N). As the embryo grows the giant cells become stretched, and some of the secondaries are replaced by cell division in the underlying cone tissue (Ilgren, 1983; M.H. Kaufman, 1983). These secondary giant cells are rarely as large as the primaries and grade into the cells of the trophospongial layer which form a meshwork through which the maternal blood flows. Unlike the adjacent labyrinthine layer there is no fetal vascularization and the trophospongium has a fine structure very different from the giant cells, but both are of the layer I population.

Layers II and III are dependent on the fetus for continued growth and maintenance, since fetectomy at 12 dpc results in their rapid degeneration together with the fetal villi. However, layer I continues to grow after fetectomy as a residual meshwork in which

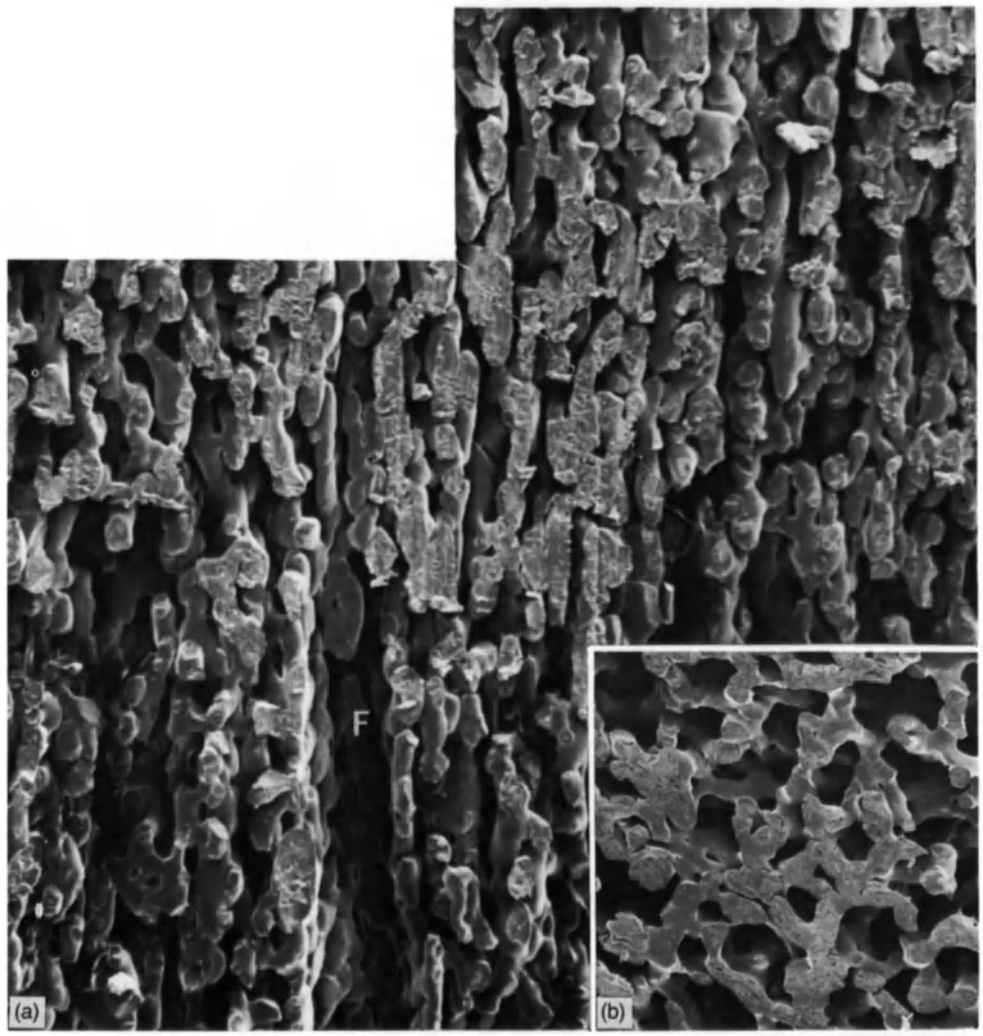


Figure 4.78 Haemochorial rat placenta. Corrosion casts of the maternal labyrinthine blood spaces. The labyrinthine architecture is elegantly demonstrated in (a) with a predominant direction of flow from the chorionic plate (top of micrograph) to the myometrium. (b) At 18 dpc the maternal blood spaces are smaller and there is no obvious direction in the structure. F, space occupied *in vivo* by the fetal vessels and membranes. (a) 22 dpc, × 150. (b) 18 dpc, × 150. (From Christofferson and Nillson, 1988.)

the maternal blood circulates (Davies and Glasser, 1968).

Haemodichorial: rabbit, *Oryctolagus cuniculus*

Oestrous cycle: none
Ovulation: on coital stimulation
Uterus: duplex
Litter: 8–12
Gestation: 30–32 days
Implantation: superficial, antimesometrial
Amniogenesis: folding
Yolk sac: (1) Large bilaminar non-vascular yolk sac persists until 16 dpc, then finally ruptures. Consists of giant trophectoderm cells, a basement membrane (much thinner

than the equivalent Reichert's membrane in the rodents) and endoderm.

(2) Inverted yolk sac placenta, vascularized villus folds adjacent to embryo. Active in uptake of macromolecules, especially immunoglobulins, during pregnancy.

Chorioallantois: definitive placenta vascularized by mesoderm of allantoic sac

Shape: discoid, labyrinthine internal structure

Decidual development: considerable

Interhaemal membrane: haemodichorial

The early conceptus development is very different from the rodents, but differentiation of the yolk sac and chorioallantoic placenta is essentially similar (Amoroso, 1952).

There is considerable blastocyst swelling prior to central implantation between 7 and 8 dpc (Figure 4.79).

Even spacing of blastocysts is achieved by the usual uterine muscle contractions refined eventually by interaction with the expanding blastocysts. Sea urchin eggs introduced into the pseudopregnant uterus are also evenly distributed although they are much smaller than the blastocyst (Boving, 1971).

Changes in the uterine epithelial glycocalyx at implantation are detailed in T.L. Anderson and Hoffman (1984) and T.L. Anderson *et al.* (1986). The first cellular changes at implantation are fusion between apposed syncytial trophectodermal knobs and cellular uterine epithelium, initially at the antimesometrial side and subsequently over the mesometrial side (where the chorioallantoic placenta eventually forms) (Larsen, 1961; Enders and Schlafke, 1971a). The uterine epithelium then fuses into syncytial plaques, each with a fairly constant number of nuclei and characteristic ultrastructure. This change also occurs in pseudopregnancy,

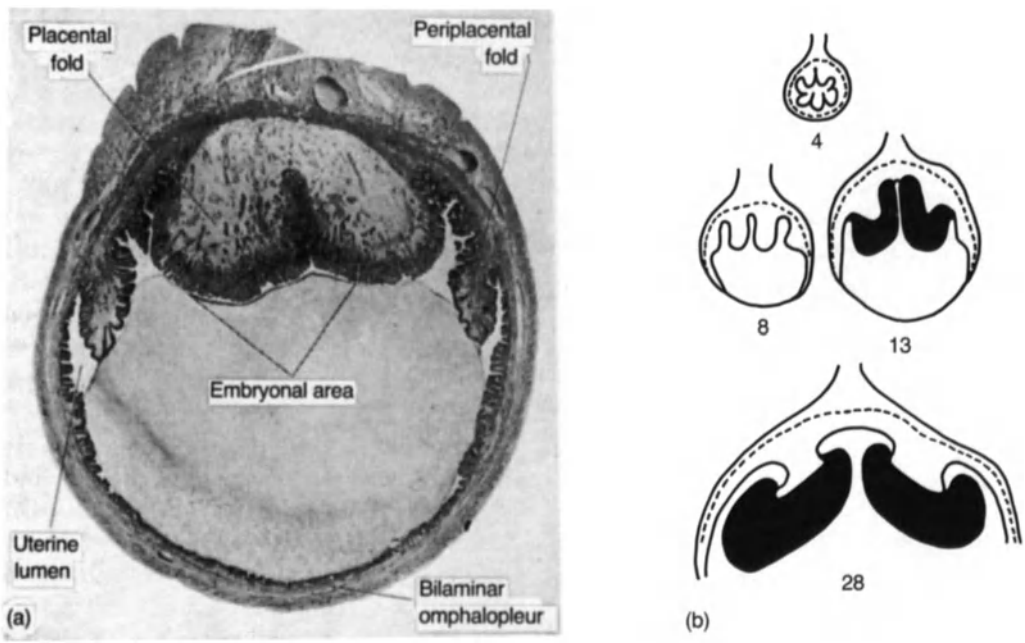


Figure 4.79 Haemochorial placenta of the rabbit. The blastocyst swells considerably before implantation (a). The chorioallantoic placenta develops mesometrially in two cotyledonary masses which increase considerably in extent during pregnancy, as shown diagrammatically in (b) (drawn actual size). (a) 8 dpc, × 7. (b) Traced from Hafez and Tsutsumi, 1966; the numbers indicate dpc; term = 30/32.

and can be induced with the correct hormonal regimen with no conceptus present (Davies and Hoffman, 1975; Davies and Davenport, 1979).

Over the antimesometrial half of the uterus the fetomaternal hybrid syncytiotrophoblast displaces the uterine epithelial syncytial plaques which degenerate into symplasmic masses which are eventually sloughed and removed by phagocytosis. In the absence of a blastocyst or physical trauma the syncytial plaques disappear in a much more controlled fashion. Reversion of individual multinucleate cytoplasmic areas to single uninucleate cells has been claimed (Busch *et al.*, 1986). However, restoration of the uninucleate epithelium seems more likely to be by proliferation of residual uninucleates as in ruminant intercotyledonary areas.

The number of fetal nuclei incorporated into the maternal syncytiotrophoblastic knobs is not known, nor is their fate. Larsen (1961) says they 'dissolve' but offers no pictorial evidence for this. The (feto?)maternal syncytiotrophoblastic knobs which penetrate the antimesometrial uterine epithelium basement membrane seem to be directly involved (Blackburn *et al.*, 1989) in the production of the uninucleate giant cells which form all round the lateral and antimesometrial aspects of the conceptus (Amoroso, 1952). Yolk sac development, with an antimesometrial bilaminar segment which ruptures at 16 dpc, leaving a fully inverted yolk sac placenta, is essentially similar to the rodents.

At the mesometrial pole once the amniotic folds have fused, the trophectoderm over the embryo invades and displaces the uterine epithelial syncytial plaques and subsequently penetrates the maternal endothelium as it did over the antimesometrial region (Hoffman *et al.*, 1990a,b). The invading fetal tissue does not form any structure equivalent to the ectoplacental cone in rodents but presents a broad syncytial front toward the decidualizing maternal tissue, backed by and presumably formed from the cellular cytotrophoblast (Figure 4.79). Thus, as soon as the fetal processes reach the maternal blood spaces they have the haemodichorial structure, layer I syncytial, layer II cellular, which characterizes the definitive placenta (Enders, 1965; Enders and Schlafke, 1971a). The allantoic vesicle has by now (9 dpc) reached the mesometrial trophectoderm and it then actively vascularizes the extending front of fetal tissue. As in the rodents the larger arteries are invaded by trophectoderm (Pijnenborg *et al.*, 1981) but persist as channels running through the full depth of the forming labyrinthine haemodichorial placenta to supply maternal blood to the subchorial lake at the base of the fetal placenta (Figure 4.72) (Hafez and Tsutsumi, 1966; Carter *et al.*, 1971). From here it flows in the newly formed fetal tissue channels and drains via maternal veins. Considering the final dimensions, the placenta must grow by extension of the fetal tissue just as much as by erosion of the endometrium (Figures 4.79 and 4.80).

The definitive placenta does not have quite the same gross sequence of maternal decidua/fetal giant cells/fetal trophospongium (no fetal vascularization)/fetal labyrinth as do the rodents since there are few, if any, giant cells in this area in the rabbit. There is no evidence for any placental lactogen production either. The haemodichorial interhaemal membrane persists but attenuates gradually to term (Samuel *et al.*, 1975). Layer II is normally much thinner than I, but both vary considerably (Figure 4.81a and b) and can be equally thin (Figure 4.81c).

They are frequently connected by small desmosomes and gap junctions (Figure 4.81b). The ultrastructure of the spongiotrophoblast is similar to that in layer I of the labyrinth but quite different from that in the adjacent giant cells (Figure 4.82).

Since the maternal blood flows back from the fetal subchorial lake and the fetal from the opposite maternal side of the placenta, the flows are grossly countercurrent. This does not imply that the fetal blood capillaries run

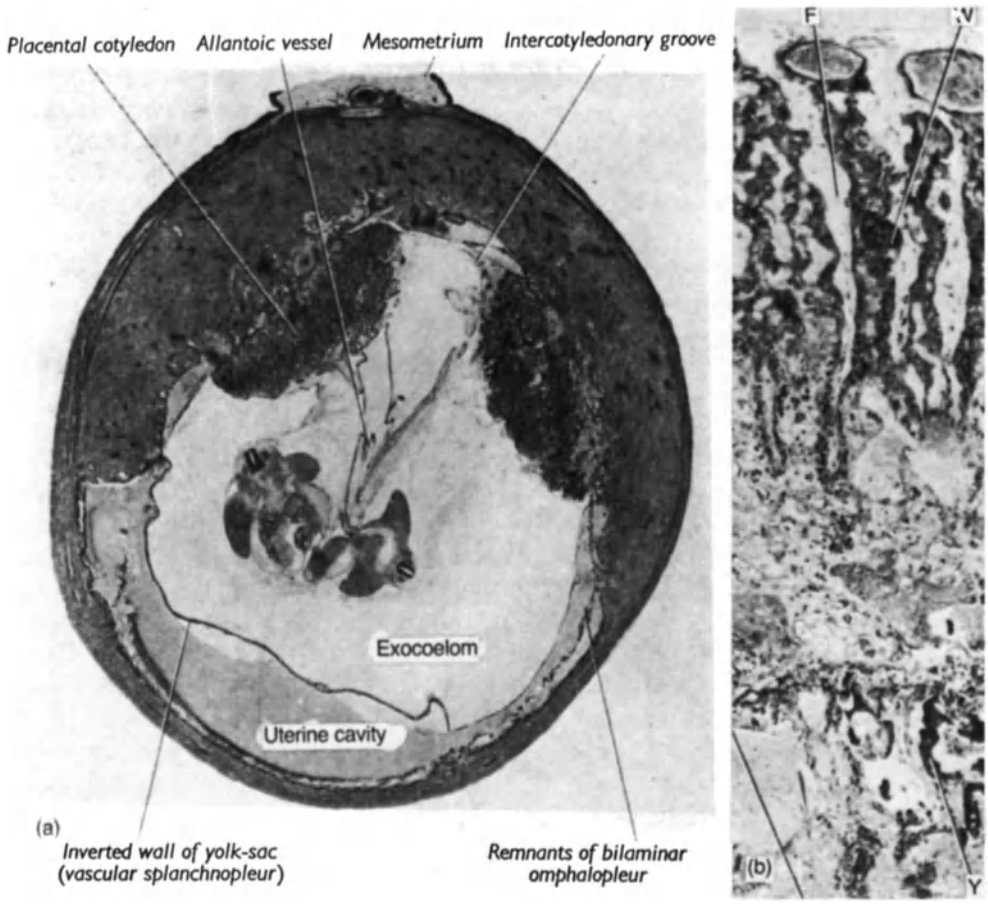


Figure 4.80 Haemochorial placenta of the rabbit. (a) Early development of the two placental cotyledons; the histological structure of this 12 dpc placenta is shown in (b). The fetal villi (F) and the maternal blood sinuses they enclose (W) increase in length continuously so that invasion of the maternal endometrium only accounts for a small proportion of total final depth of the placenta. Note the symplasmic remnant (Y) of a gland in the junctional zone. (a) 12 dpc, × 6.5. (b) 12 dpc, × 6.5.

parallel to the maternal channels through the entire depth of the placenta, but rather that the two blood flows (microflows, see Figure 4.8) maintain a very close relationship for a sufficient distance to allow efficient gaseous exchange. This capillary length is what Mossman (1965) refers to as an 'exchange distance'.

Just before parturition (28 dpc) a discrete thin lamina of uninucleate decidual cells forms on the myometrial side of the decidua basalis (Mossman, 1987). This forms the zone of separation of the placental stalk, which is considerably narrowed at parturition. These two processes, together with contraction of the myometrium, reduce the placental scar to minimum dimensions (Amoroso, 1952). The myometrial contractions are considerably enhanced at this time by the increase in frequency of gap junctions between individual smooth muscle cells (Cole and Garfield, 1989).

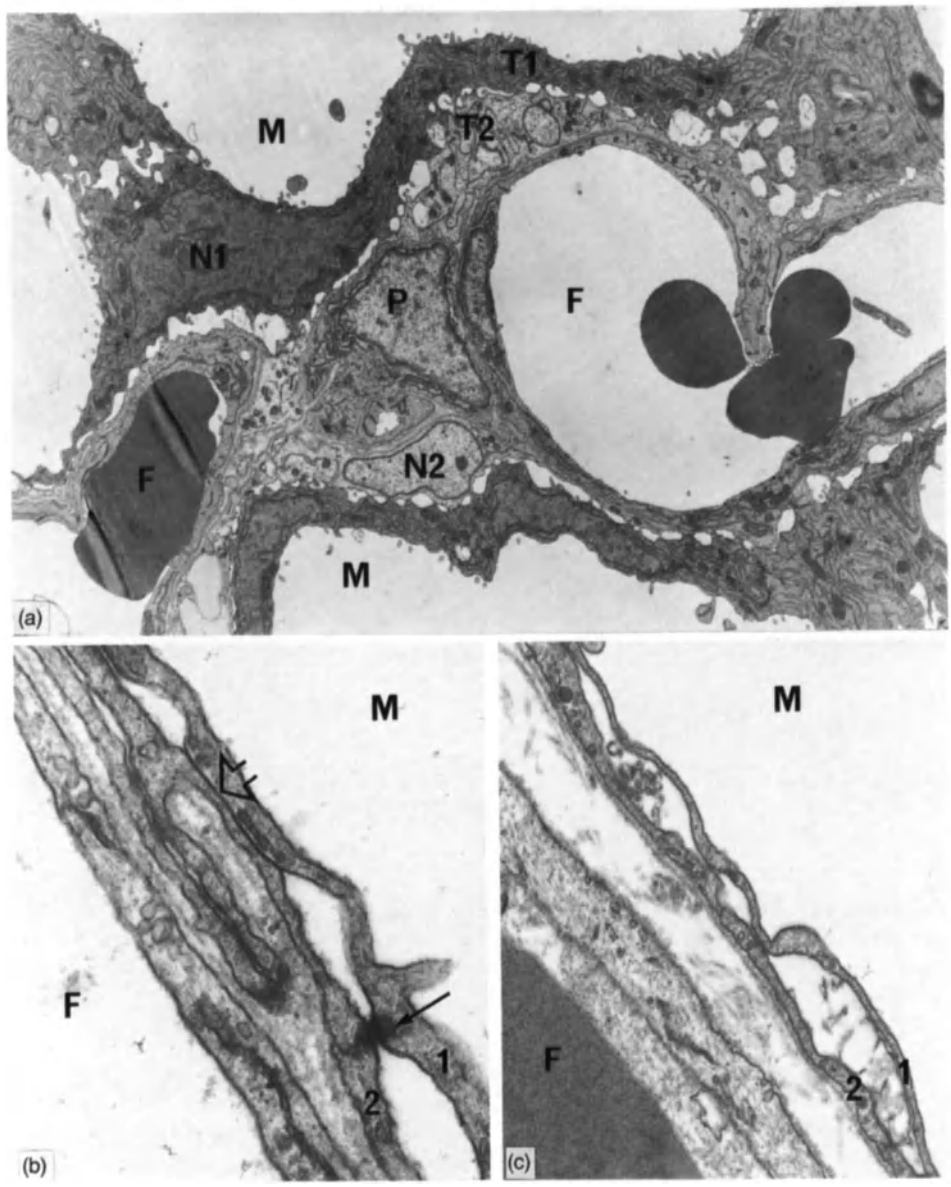


Figure 4.81 Haemodichorial rabbit placenta. (a) Definitive labyrinth structure. The maternal blood space (M) is bounded by two layers of trophectoderm (T1, T2), each of which show nuclei (N1, N2); T1 is a syncytium, T2 unicellular. T1 has far more rough endoplasmic reticulum (asterisks) than T2, which is considerably thinner. F, fetal blood capillaries; P, fetal pericyte. (b) There are frequent gap (open arrow) and small desmosomal (small arrow) junctions between layers 1 and 2. (c) Although layer 2 is usually thinner both layers frequently reduce to an equivalent exiguous extent. (a) 22 dpc, × 4000. (b) 22 dpc, × 35 000. (c) 22 dpc, × 15 000.

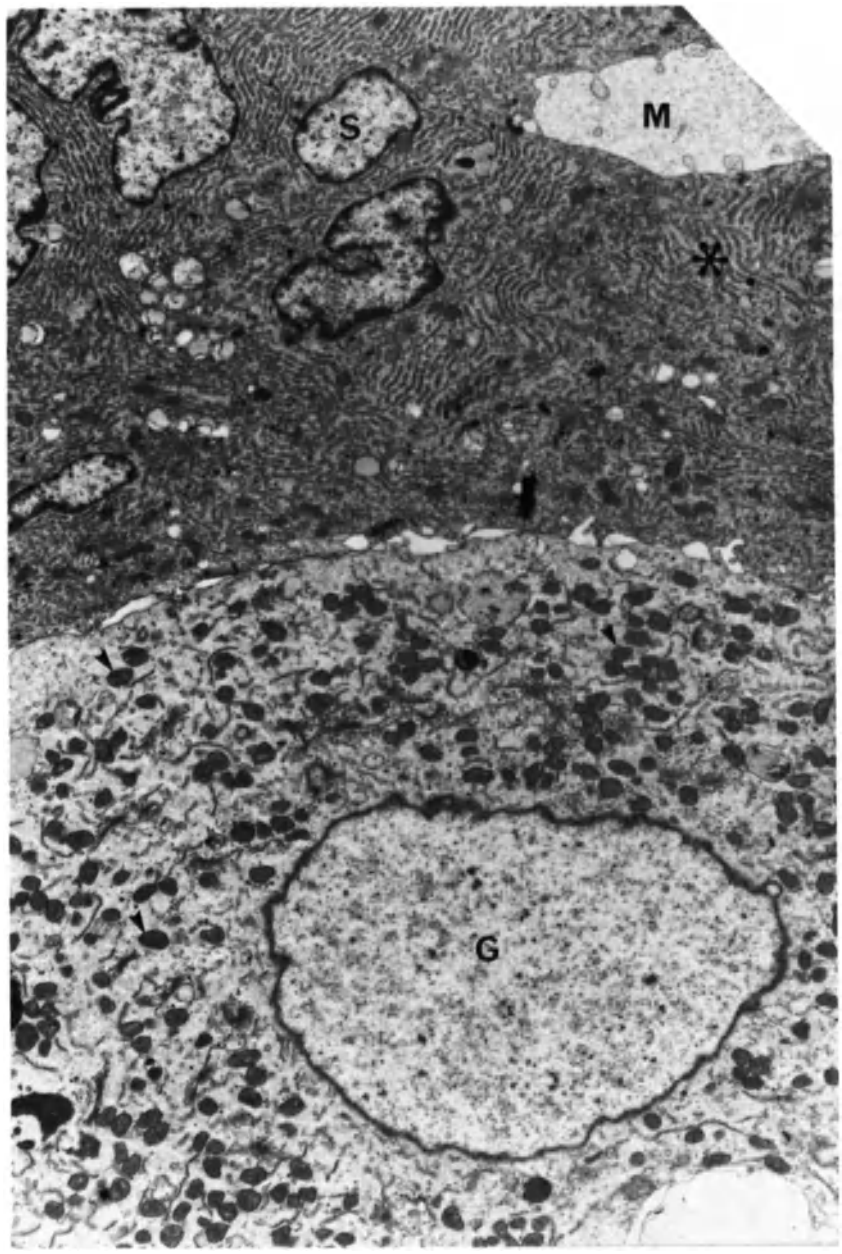


Figure 4.82 Haemodichorial rabbit placenta. The fine structure of the labyrinthine layer T1 on Figure 4.81 is similar to that in the spongiotrophoblast (S) shown here with massive arrays of endoplasmic reticulum (asterisk). The giant cells (G) have a very different ultrastructure with larger and more numerous mitochondria (arrowheads) and scattered individual strands of endoplasmic reticulum. M, maternal blood space. 22 dpc, × 7500.

Haemomonochorial: human, *Homo sapiens*

Menstrual cycle: 28 days
Ovulation: spontaneous
Uterus: simplex
Litter: 1–3
Implantation: interstitial, antimesometrial
Gestation: 270 days
Amniogenesis: cavitation
Yolk sac: The endoderm develops in an unusual manner and initially forms a latticework in the lumen of the blastocyst, only secondarily forming a sac (Luckett, 1978). This never becomes significantly larger or vascularized (Figure 4.2).
Chorioallantois: this forms the definitive placenta
Shape: discoid
Decidual development: considerable
Interhaemal membrane: haemomonochorial

The morula enters the uterus on 4–5 dpc transforms to a blastocyst, loses its zona pellucida and normally implants in the midline of the upper part of the posterior wall on 6 dpc. It is not clear how it is so positioned but uterine closure ensures no significant further movement. Implantation occurs prior to any blastocyst swelling, the cellular trophectoderm produces a syncytial cap which rapidly intrudes through the uterine epithelium and the whole blastocyst passes into the endometrium. The details of this process are not understood. Once the conceptus is established in the endometrium (6–7 dpc, Figure 4.83a and b) active proliferation of the cytotrophoblast produces the basis for rapid syncytium formation (Aplin, 1991).

No nuclear division has ever been reported in the human syncytiotrophoblast, but there are several autoradiographic and morphological studies showing that the cytotrophoblast is the precursor of the syncytiotrophoblast throughout pregnancy (Richart, 1961; Galton, 1962; Tao and Hertig, 1965; Tedde and Tedde-Piras, 1978; Kurman *et al.*, 1984). The syncytiotrophoblast thickens all around the conceptus and on 10–11 dpc lacunae fringed with microvilli develop within its bulk. It is actively invasive and soon breaches the maternal capillaries to start the flow of maternal blood into the lacunae (Figure 4.83c) (Boyd and Hamilton, 1970; Enders, 1989).

Columns of cytotrophoblast cells then proliferate into and through the syncytium to initiate the fetal villi over the whole conceptus (Figure 4.83d). Subsequently, the villi grow preferentially toward the best maternal blood supply so that villus development becomes asymmetric and eventually restricted to an antimesometrial discoid area (Figures 4.84 and 4.85).

The allantoic vesicle is rudimentary in the human but there is a vigorous growth of allantoic mesoderm to vascularize the developing placental villi, which by 20 dpc have grown through the syncytium and spread laterally to form a cellular cytotrophoblastic shell around the conceptus (Figure 4.83d). This is actively phagocytic towards the decidual cells against which it is apposed, and it also produces cells which migrate deep into the endometrium (Aplin, 1991). The maternal spiral arteries are also invaded by cytotrophoblast, which removes and replaces the endothelium and pericytes to produce by mid-pregnancy widened channels completely unresponsive to normal endothelial agonists, through which maternal blood is supplied to the endometrium (Pijnenborg *et al.*, 1983). There is some evidence that if this process is inhibited or blocked there are considerable problems of insufficient blood flow resulting in the hypertensive disorders of pregnancy (Robertson *et al.*, 1986; Shanklin and Sibai, 1989).

As soon as the cytotrophoblast shell has formed around the conceptus (21 dpc) a layer of fibrinoid material is developed between the decidua and the shell (P. Kaufmann, 1981b; Aplin 1991). In man the decidual cells are not packed closely together as in rodents, but are also surrounded by this fibrinoid

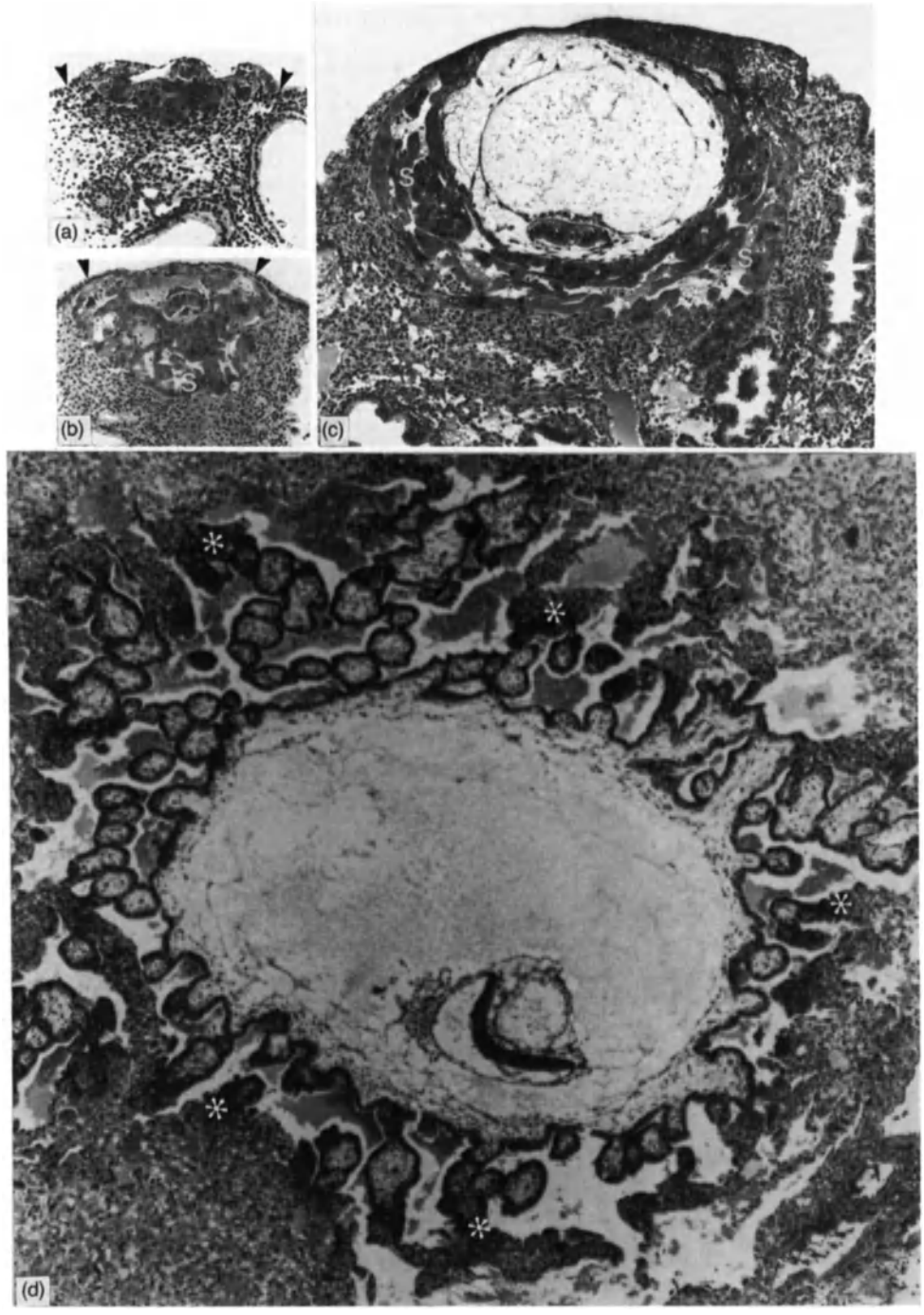


Figure 4.83 Haemochorial placenta in man. Early stages of development, all at the same magnification. (a) The blastocyst passes through the uterine eithelium (a and b, arrowheads) and (b) establishes itself in the endometrium. (b and c) The syncytiotrophoblast (S) forms the initial boundary around the conceptus but (d) with the formation of the chorionic villi all around the periphery a cellular cytotrophoblastic shell (asterisks) develops around the conceptus and facilitates further villus growth. (a) 7½ dpc, × 55. (b) 9½ dpc, × 55. (c) 12 dpc, × 55. (d) 14 dpc, × 55. (From Boyd and Hamilton, 1970.)

material which they synthesize (Wewer *et al.*, 1985; Kisalus *et al.*, 1987). It is thus more likely that the two major fibrinoid layers, Rohr's and Nitabuch's 'striae', are maternal rather than fetal products. They are in no sense continuous barriers between the fetal and maternal compartments since cytotrophoblast cells penetrate past them as far as the myometrium in some cases (Loke *et al.*, 1986). These cells, which may develop into syncytial plaques by fusion, are referred to as 'extravillous' trophoblast (the term includes endovascular trophoblast) (Schiebler and Kaufmann, 1981). They have the same monoclonal antibody plasmalemma markers but lack the hormonal marker hCG typical of villous syncytiotrophoblast (Bulmer *et al.*, 1984; Gosseye and Fox, 1984; Loke *et al.*, 1986; Robertson *et al.*, 1986). Extravillous trophoblast also expresses MHC class I and HLA G antigens, which are absent from villous syncytiotrophoblast (Redman *et al.*, 1984; Loke and King, 1989; Hunt and Hsi, 1990; Hunt *et al.*, 1990).

As with the other haemochorial placentas discussed above, it is clear that most of the villus elongation is due to expansion of the fetal tissue after the initial erosion of the maternal tissue has occurred, since a considerable thinning of endometrial substance must also be caused by the stretching needed to accommodate the increase in conceptus size. Mossman (1987) has evidence that there is very little increase in thickness of the cytotrophoblast shell or the decidua from as early as 25 dpc to term, but the villi increase in length eightfold in that time.

One clue as to the depth of fetal erosion may be the height of the shallow ridges (septae) of maternal tissue which are covered with cytotrophoblast shell (Figures 4.85c and 4.88). They delineate the aggregates of fetal villi which cluster around the points at which spiral arterial channels enter through the basal plate (cytotrophoblastic shell plus Nitabuch's stria). They are presumably produced because there are no villi over the septal region of the basal plate and therefore less erosion.

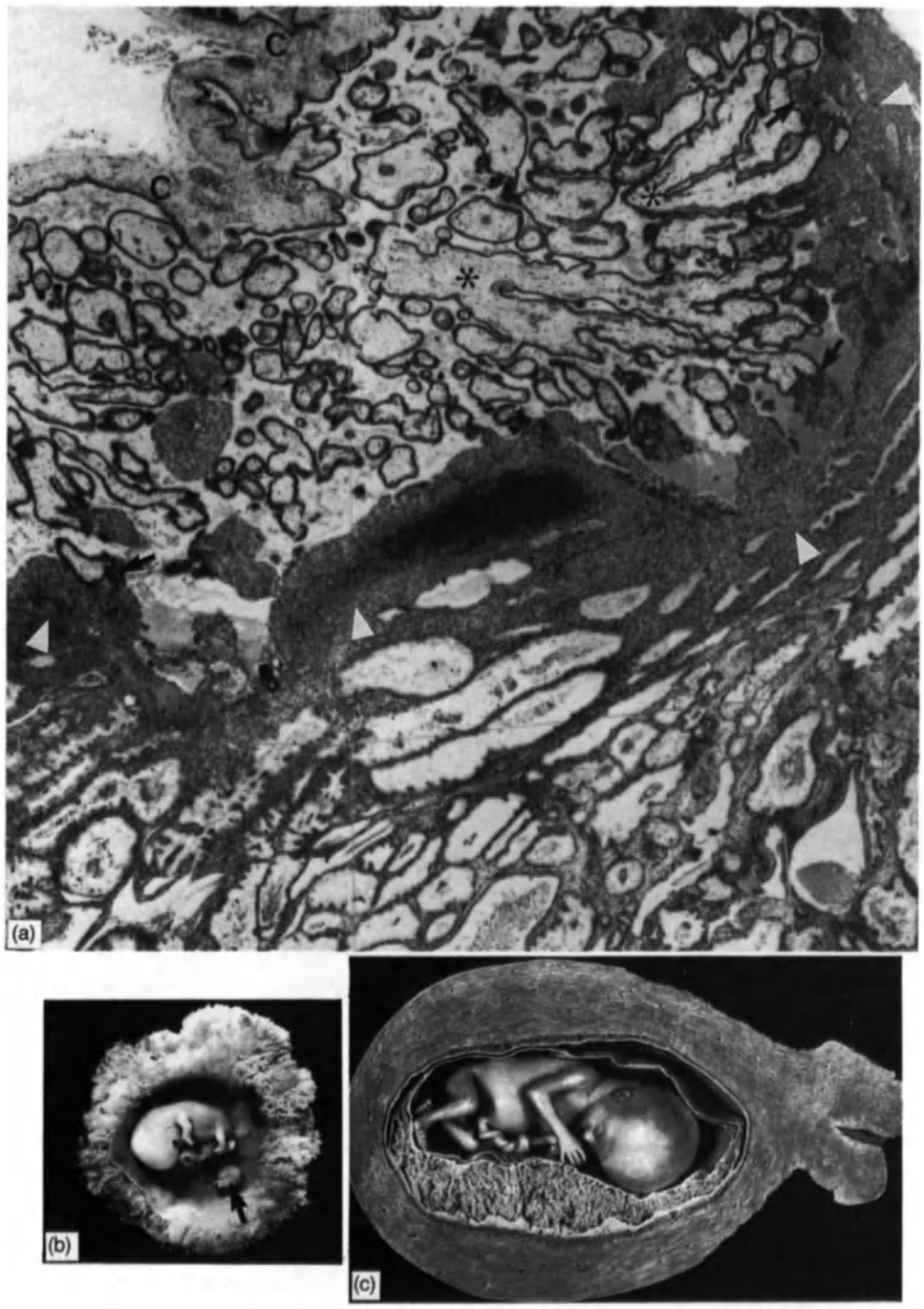
An individual villus consists initially of solid cytotrophoblast, but this is invaded by the allantoic vasculature by 18 dpc. The young stem villus has a cytotrophoblastic tip at its insertion into the cytotrophoblastic shell (Figure 4.84a). Behind this, where the villus is bathed in maternal blood, the cytotrophoblast is covered by a continuous syncytiotrophoblast layer.

The villus elongates and branches, becoming tree like, usually retaining at least one connection via a terminal branch to the basal plate as well as its stem inserted into the chorionic plate (Figures 4.84 and 4.90) (Boyd and Hamilton, 1970; P. Kaufmann, 1981b; Burton, 1987, 1990; Mossman, 1987; Castellucci *et al.*, 1990).

There are three major levels of villus organization, primary or stem, secondary and terminal. Morphometrically there is a progressive loss of villus connective tissue, an increase in the volume occupied in the villus core by fetal blood vessels and a decrease in thickness of the syncytiotrophoblast over the closely adjacent fetal vessels (Kaufmann *et al.*, 1985) (Figures 4.86 and 4.87).

This reaches an extreme in the so-called 'vasculosyncytial' membrane in the terminal villi where the distance from the maternal to fetal blood is reduced to less than 3 μm over 40% of the total area of the terminal villus whose volume consists largely of blood vessels (Figure 4.86) (P. Kaufmann, 1985; Mayhew *et al.*, 1986) (Figures 4.86, 4.87 and 4.88).

Local variations in proliferations of the basal syncytiotrophoblast membrane are also found (Figure 4.87). In man and baboon endogenous C-type retrovirus particles have been observed budding exclusively from the basal syncytiotrophoblast plasmalemma (Kalter *et al.*, 1973). From an early stage the cytotrophoblast layer becomes discontinuous and ever more exiguous. The placenta is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(a)
(b)
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therefore considered haemomonochorial with only the syncytiotrophoblast forming a significant boundary layer (Figure 4.98b). For a recent review of human placental ultrastructure see Jones and Fox (1991).

The increase in area of the interhaemal membrane depends on cytotrophoblast division and fusion to form syncytiotrophoblast. Intermediate stages of this process have been reported (Kurman *et al.*, 1984). The interhaemal membrane has been shown to grow continuously throughout pregnancy so the cytotrophoblast certainly must survive until term. Placental weight and DNA content increase right up to parturition (Sands and Dobbing, 1985) and division of cytotrophoblast has been demonstrated in tissue cultures of term placenta (Kliman *et al.*, 1986). Simpson *et al.* (1992) have also recently shown morphometrically that the ratio of cytotrophoblast nuclei to syncytiotrophoblast nuclei is constant throughout gestation with no decrease near term.

The definitive placenta consists of groups (or lobules) of 3–5 stem villi bounded by septa (Figures 4.85c, 4.88, 4.89 and 4.90).

Latex injection studies suggest that there is a loose or hollow centre to each lobule and that a maternal spiral arterial channel supplies this hollow centre from its base (Figures 4.89 and 4.90) (Wigglesworth, 1969). Angiographic studies indicate that maternal blood spurts as a 'puff' into each hollow lobule from each spiral arterial channel (Figure 4.89c) and drains by venous exits at the sides and bases of the septa (Figure 4.88) (Ramsey and Donner, 1980). Since the villi are flaccid and clumped in any delivered placenta it is difficult to envisage the three-dimensional architecture during maternal blood flow. A possible analogy might be water flow through fish gills, with the gill filaments equivalent to the secondary and terminal placental villi. These would be separated to capillary dimensions by each 'puff' of maternal blood, closing like a fish gill to prevent backflow. When the blood reaches the interlobular space it would flow out of the wide venous channels in the basal plate and the septa. There is some autoradiographic (Geier *et al.*, 1975), ultrastructural (Critchley and Burton, 1987) and histochemical evidence (Schuhmann, 1982) for significant differences between the villous structure at the hollow centre of the lobule and the periphery, supporting the concept of maternal blood streaming out from the centre. However, the disposition of fetal blood vessels in the terminal villi makes countercurrent flow between fetal and maternal blood channels only one of many possible relationships. The overall result is probably mixed villous flow ('network current' flow, see Figure 4.8) (P. Kaufmann *et al.*, 1985) (Figure 4.88).

At implantation the conceptus does not embed itself very deeply into the endometrium and so the development of villi over the antimesometrial portion is considerably restricted because of the poor maternal blood supply to the thin decidua capsularis and the uterine epithelium boundary (Figures 4.84 and 4.88). In this paraplacental area the original fetal vascularization of the antimesometrial chorionic membrane is lost, as is the

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Figure 4.84 Haemochorial placenta in man. (a) The chorionic villi increase in length and degree of branching (asterisks) as pregnancy progresses. The villi insert into the cytotrophoblastic shell (arrows) above a zone of decidual cells (white arrowheads). The endometrial glands (bottom third of figure) are eroded and distorted by the growth of the placental disc but they still appear to be actively secreting material. C, chorionic plate, (b and c) Halved conceptuses (c still *in utero*) illustrate the reduction in trophectodermal villi from a uniform halo to a localized disc between 55 dpc and 80 dpc. Note the vestigial yolk sac (arrow) in (b). (a) 25 dpc, × 10. (b) 55 dpc, × 0.5. (c) 80 dpc, × 0.5. (From Boyd and Hamilton, 1970.)

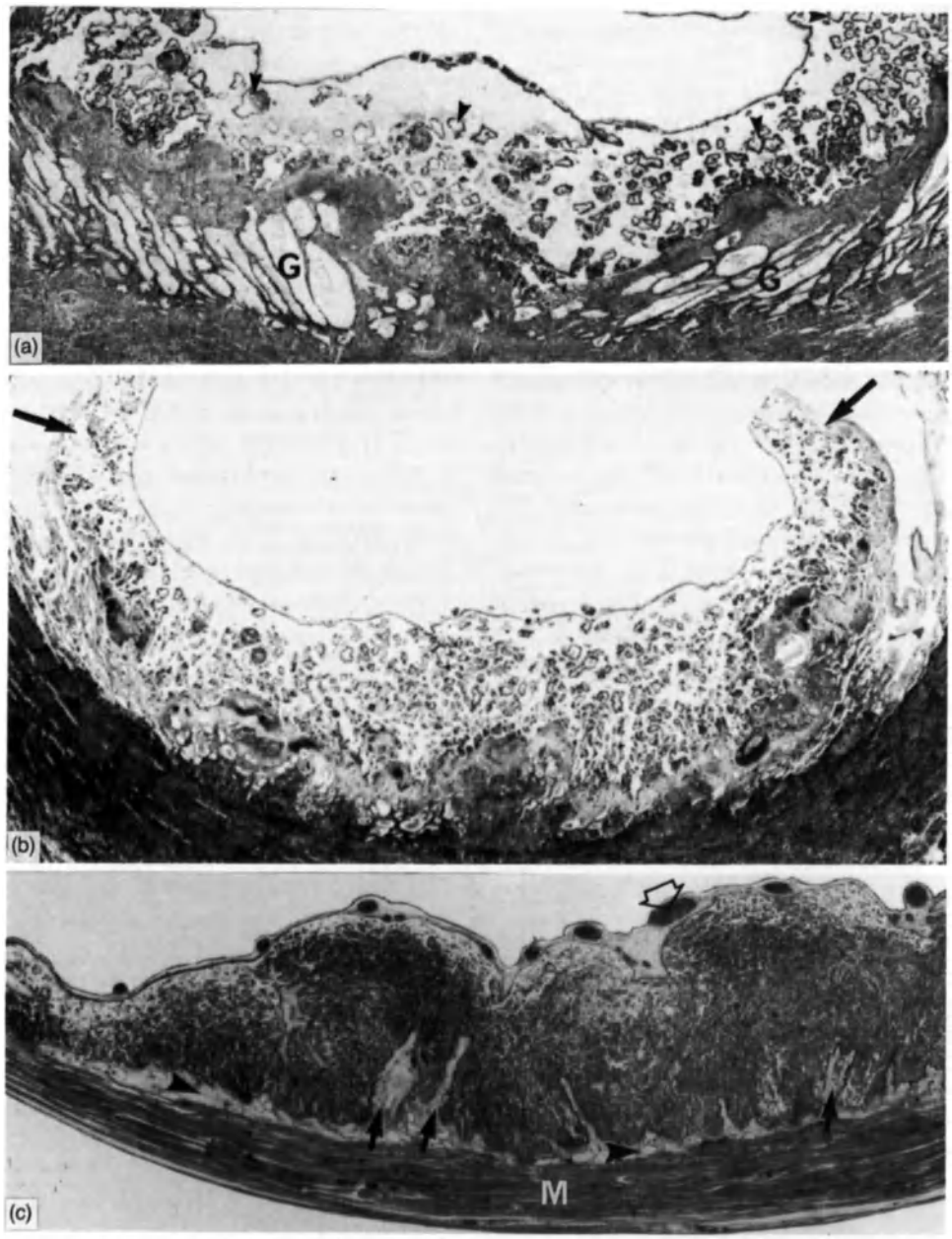


Figure 4.85 Haemochorial placenta in man. Development of the definitive placental disc. (a) The initially broad villi (arrowheads) are arranged evenly around the conceptus at 32 dpc and the basal uterine glands (G) are still extensive. At 42 dpc, (b) the villi are thinner and more frequent in the placental disc area, and have virtually crowded out the uterine glands basally, but are regressing outside the placental disc (arrows). (c) By 140 dpc the villi are much more extensive and closely packed. (This micrograph is only one quarter of the magnification of a and b). Septa (arrows) are now obvious. They are continuous with the thin decidual layer (arrowheads) which lies above the myometrium (M). The septa divide the placental disc into lobules or 'cotyledons' (see Figures 4.63 and 4.64). Open arrow, fetal blood vessel in chorionic plate. (a) 32 dpc, × 6. (b) 42 dpc, × 6. (c) 140 dpc, × 1.5. (From Boyd and Hamilton, 1970.)

syncytiotrophoblast layer, leaving a multilayered cellular chorion with an avascular mesodermal connective tissue layer inside. As it enlarges the amnion joins with this to form an amniochorionic membrane. By the fifth month the growth of the fetus has stretched and obliterated the decidua capsularis and its overlying uterine epithelium. The uterine epithelium over the decidua parietalis is also lost at this time so that the chorion is now apposed directly to the decidua parietalis (see Figure 4.88). This complex membrane (or 'chorion laeve') persists throughout pregnancy though considerably thinned by growth of the fetus. There is some immunohistochemical evidence for prolactin production by the capsularis decidual cells so the membrane is not physiologically inert (Meuris *et al.*, 1980). However, no areolar structures develop since there is no significant glandular presence in the paraplacental region (Boyd and Hamilton, 1970). There are gland remnants embedded in the basal decidua which are synthetically active at term (Nelson *et al.*, 1986).

Since the fetal trophoblast is bathed in maternal blood from the earliest stage of placental development there is no need for any specialized haemophagous zone and iron is probably transported from the maternal blood using the transferrin binding protein. A specialized plasmalemmal site for iron binding has been suggested (Ockleford and Menon, 1977). There is no evidence for any significant phagocytosis of red blood cells by the fetal syncytiotrophoblast. Human chorionic gonadotropin (hCG) and placental lactogen hormones are synthesized and stored in different granules in the syncytium (Figure 4.91) (Johnson and Wooding, 1988; Morrish *et al.*, 1988; Billingsley and Wooding, 1990), which also contains immunoreactivity for α-, β- and γ-interferon (Bulmer *et al.*, 1990), corticosteroid-releasing hormone (Riley *et al.*, 1991), EGF (Hofmann *et al.*, 1991) and IGF-1 and -2 and their binding proteins (Zhou and Bondy, 1992; D.J. Hill *et al.*, 1993).

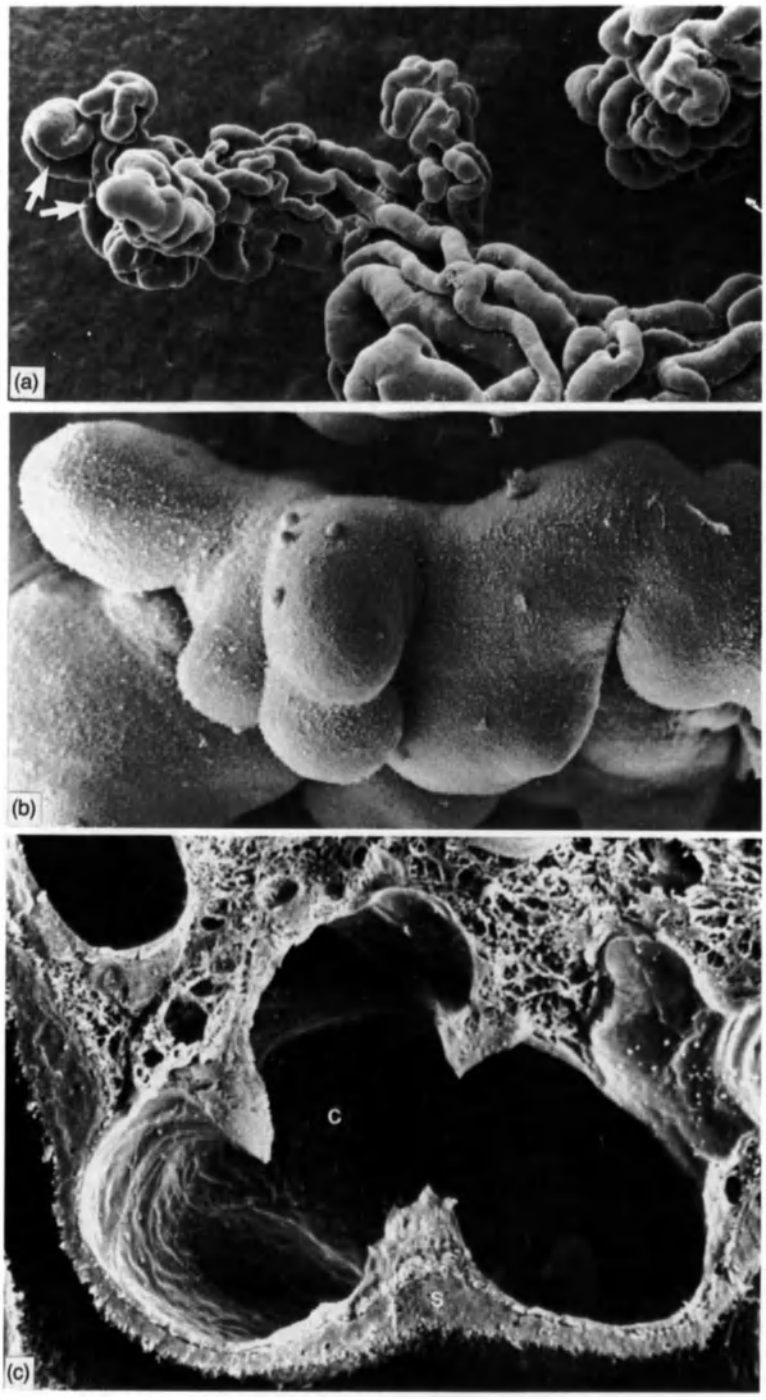
Unlike most animals, there is no fall in progesterone levels prior to parturition in man, nor is there any change in relaxin levels, which anyway are much lower than they were during the first 3 months of pregnancy (see Sherwood, 1988, for an excellent review). Labour in man is also unusual in that it evolves gradually over a period of many days without an abrupt onset as in most species studied. Oxytocin is said to play no significant role until the second (expulsive) stage of labour when it ensures retraction of the uterus and prevents excessive blood loss (Casey *et al.*, 1983). The trigger for human parturition remains controversial but may involve oestrogen precursor production from the fetal adrenal and prostaglandin secretion by amnion and the parietal chorion (Liggins, 1981; Casey *et al.*, 1983).

Other anthropoids: 1. Apes and Old World monkeys

As far as they have been investigated the development and definitive placentation of man and the great apes (chimpanzees, gorillas, orangutans) are virtually identical. The Old World monkeys (catarrhines) have a very different implantation and development, although the definitive structure of the placenta is almost indistinguishable at macroscopic and ultrastructural levels (Amoroso, 1952; Ramsey, 1982; B.F. King, 1986).

The best-studied Old World monkey is the macaque or rhesus, *Macacus rhesus*. Details of the reproduction are identical to those for *Homo* (section 4.3.6d) save that the gestation is only 160 days, the placenta is bidiscoid, implantation is superficial and there is little stromal decidual development but a considerable proliferation of uterine epithelial plaque (Figures 4.92 and 4.93) (Wislocki and Streeter, 1938; Enders *et al.*, 1985).

Plaque only persists for the first month of pregnancy and may be one reason for the limited invasion of the endometrium. It can also be produced by scratch trauma as a prel-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ude to deciduoma formation (Rossman, 1940). The invasive syncytiotrophoblast of the adherent blastocyst needs only to penetrate into the superficial, greatly hypertrophied endometrial blood vessels to establish a good blood supply (Figure 4.93) (Enders, 1989; Enders and King, 1991; Enders and Welsh, 1993). Once this is accomplished extensive exclusively fetal villus growth can produce the definitive placenta (Figure 4.94) with a villus structure equivalent to man (compare Figures 4.84a and 4.94c).

There is no need for any deeper invasion of the endometrium since the maternal blood supply is always at the base of the placenta, as in the human. Fetal cytotrophoblast does invade the spiral arteries replacing the endothelium as far back as the myometrium but there is less erosion of the arterial elastic sheath than in man. This is probably because the rhesus cytotrophoblastic shell, although it does synthesize connective tissue components (Blankenship *et al.*, 1992), is not at all invasive so there are very few if any cytotrophoblast-derived cells in the endometrial connective tissue to attack and erode the artery wall to the extent seen in man (Ramsey *et al.*, 1976). There seems to be no simple relationship between epithelial plaque or decidua formation and the depth of implantation since the baboon has superficial implantation but no epithelial plaque and no obvious decidual development; the macaque has superficial implantation, extensive plaque and limited decidual development; and man has interstitial development, no epithelial plaque and massive decidualization (Ramsey *et al.*, 1976). All primates are said to have 'extensive decidua' by mid-pregnancy (Enders, 1991).

Rhesus and the African green monkey *Cercopithecus* (Owiti *et al.*, 1989) are good models for studying the functions of the **definitive** human placenta since the structures are closely similar. However, the Old World monkeys are poor models for events at implantation and in early pregnancy in humans because interstitial or superficial implantation with decidua or plaque development are such very different processes (Enders, 1989).

Other anthropoids: 2. New World monkeys

There are two families: Cebidae, including howler, squirrel and spider monkeys; and Callithricidae, the marmosets. There are only isolated studies of the cebids and all prior to the advent of the electron microscope (Wislocki, 1929, 1930; J.P. Hill, 1932). Recently studies have concentrated on the marmoset (*Callithrix jacchus*) since it is relatively easy to maintain in breeding colonies (Moore *et al.*, 1985; Merker *et al.*, 1987; C.A. Smith *et al.*, 1987).

There is no overt menstruation in New World monkeys which correlates with an absence of spiral arteries in the endometrium (Ramsey, 1982). Cyclic proliferation and degeneration of the spiral arteries is thought to be the basis of the menstruation characteristic of other anthropoids (Ramsey, 1982).

Implantation is superficial, usually with considerable epithelial plaque formation (J.P. Hill, 1932) although there is very little in marmosets (Moore *et al.*, 1985).

After attachment (11–12 dpc) the blastocyst

Figure 4.86 Haemochorial placenta of man. (a) Scanning electron micrograph of a corrosion cast of the blood capillary meshwork in the terminal villi. Note the sinusoidal swellings at the ends of the villi (arrows). (b) Scanning electron micrograph of the surface of an intact perfused terminal villus emphasizing that the villus consists almost entirely of blood capillary volume. (c) Scanning electron micrograph of a cross-fracture through a specimen similar to that in (b). Note the considerable size of the capillary (c) and the thin investment of syncytiotrophoblast (s). (a) Term placenta, × 200. (b) Term placenta, × 400. (c) Term placenta, × 1400. (From Burton, 1987.)

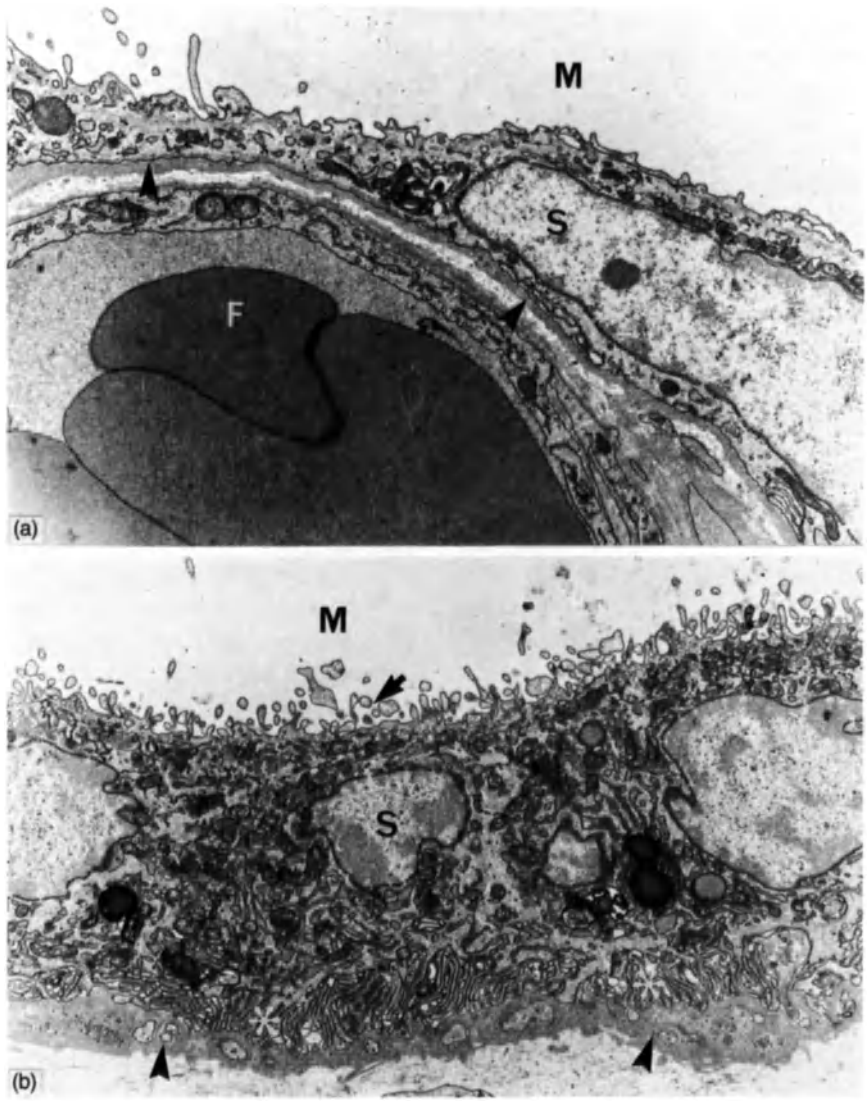


Figure 4.87 Haemochorial placenta of man. Transmission electron micrographs showing variation in local structure. (a) Over the fetal blood vessel (F) the syncytiotrophoblast layer (S) is extremely thin as is the basement membrane (arrowheads). (b) Away from the capillaries the fetal syncytium is thicker and the basal plasmalemma shows a complex elaboration (asterisks), increasing the membrane area equivalent to the microvillar forms (arrow) of the apical membrane. The basement membrane is considerably thickened (arrowheads), M, maternal blood space. (a) Term placenta, × 9000. (b) Term placenta, × 7500.

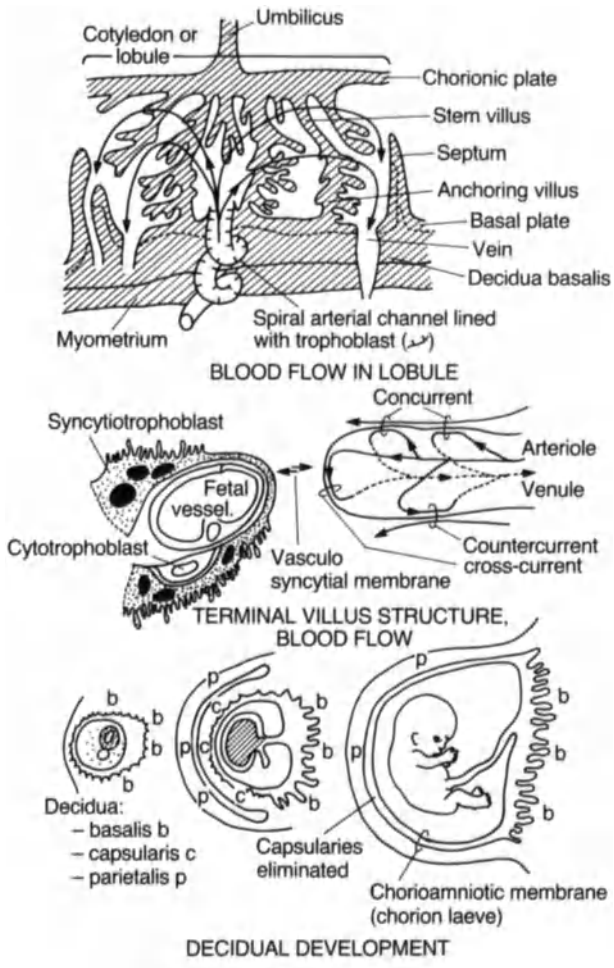


Figure 4.88 Blood flow in the definitive human placenta and development of the decidual tissues.

cytotrophoblast produces syncytium which penetrates the uterine epithelium. Once the stroma is reached the cytotrophoblast proliferates to produce a thick pad of syncytiotrophoblast which surrounds but does not penetrate the superficial maternal capillaries (23 dpc) (C.A. Smith *et al.*, 1987). By this time the epithelial plaque is regressing. The syncytiotrophoblast then develops lacunae within its bulk, but in the marmoset no maternal blood is observed within these spaces until 80 dpc, halfway through pregnancy, when the syncytiotrophoblast finally erodes the maternal endothelium. At this stage the fetal mesoderm pushes into the trophoblast layers, forming vascularized trabeculae within the meshwork of syncytium (C.A. Smith and Moore, 1990). The syncytiotrophoblast not only appears very similar ultrastructurally to that of other primates (C.A. Smith and Moore, 1990) but also has equivalent C-type retrovirus particles budding from the syncytiotrophoblast basal plasmalemma opposite the cytotrophoblast cells. The cytotrophoblast layer becomes discontinuous very early, and these fetal trabeculae are

bounded largely by syncytiotrophoblast. Further development of the placenta is by continual extension and subdivision of the trabeculae to form a complex meshwork bounded by syncytium. The trabeculae become more individual and more slender during later pregnancy (Figure 4.95) (Wislocki, 1929; 1930; Amoroso, 1952; Smith and Moore, 1990).

In the spider monkey, *Ateles*, the thickness of the placental pad does not increase significantly as the fetus grows from 50 to 180 mm (term) crown–rump length (Wislocki, 1930). The process is not villus elongation but one of the continual subdivision of a coarse trabecular meshwork to a finer one with a considerably greater surface area for fetomaternal exchange. The New World monkeys thus achieve a structural basis for a significant blood circulation much more slowly than the Old World monkeys. Possibly in compensation for this, the New World monkeys

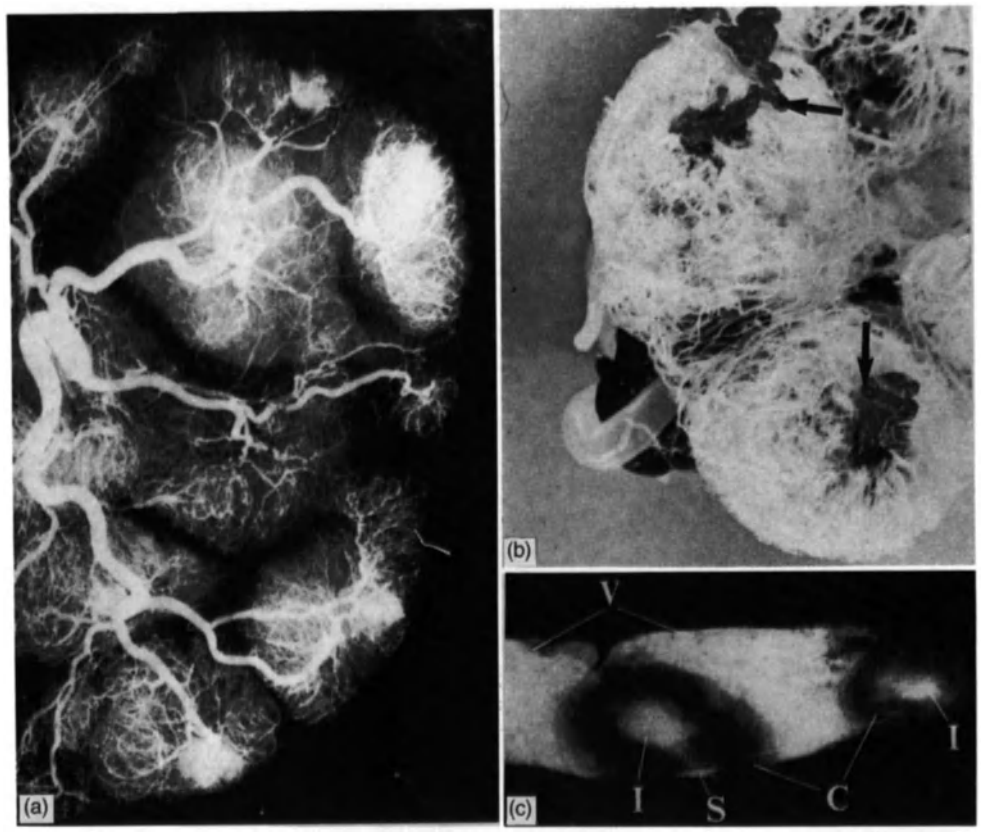


Figure 4.89 Haemochorial placenta of man. Vascular relationships. (a) Injection of the fetal circulation of a term placenta viewed from the fetal side. This illustrates the supply to individual lobules or cotyledons. (b) Latex casts of the fetal circulation (white) and of the maternal spiral arteries (black) of a term placenta viewed from the maternal side. One maternal spiral artery (arrows) enters the centre of each lobule or cotyledon. (c) Section of a term placenta injected via the spiral arteries (S) with X radio-opaque medium. The injection fills a central area (I) in the two lobules or cotyledons (C) but is excluded from the zone immediately surrounding the injection by the densely packed villi. The presence of opaque medium in the intervillous space (V) shows that it was able to pass across the villi but not to leak back. (From Wigglesworth, 1969.)

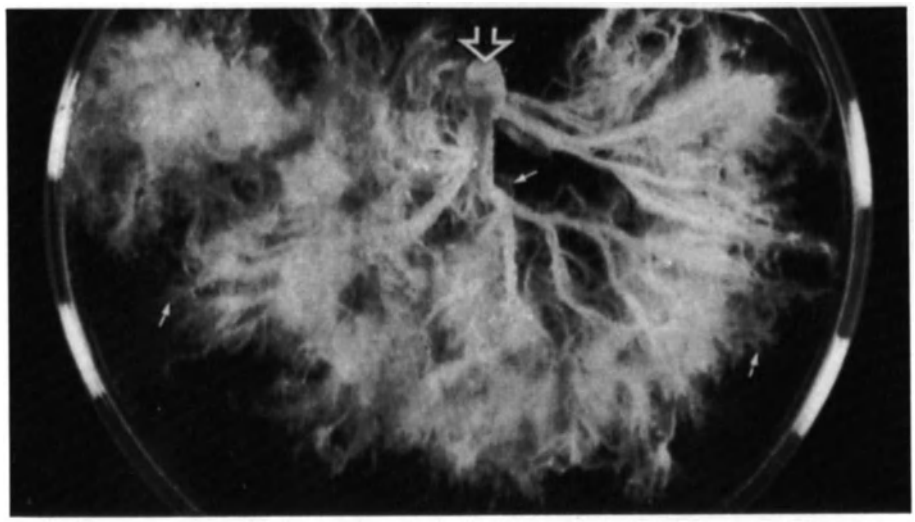


Figure 4.90 Haemochorial placenta of man. Stem villus dissected after trypsin digestion from a term placenta. The chorionic plate insertion was at the open arrow. Two or three of such villi would form one lobule or cotyledon. The finest 'twigs' (arrows) correspond to the terminal villi shown on Figure 4.86. (From Ramsey and Donner, 1980.)

show a massive uterine glandular activity throughout pregnancy (Wislocki, 1929), whereas the Old World monkeys, apes and man have very little. However, the gestation length and placental weight for equivalent-sized neonates is similar in the two groups; there is no evidence that one is more 'efficient' than the other, although the size and degree of fusion of the trabeculae or villi vary considerably (Figure 4.95).

The efficiency of the fetomaternal exchange in New World monkeys may also be influenced by the persistence, uniquely in anthropoids, of one or two wide maternal arterial channels per lobule which run right through the entire thickness of the placental disC and terminate just under the chorionic plate (Figure 4.72). These conduits have occasional openings distributed apparently randomly along their walls and at their tips. The walls of the channels consist of a continuous sheet of cuboidal epithelial cells which are assumed to be of maternal origin. These cells secrete a thick basement membrane like material around the vessels, which persist to term (Wislocki, 1930; J.P. Hill, 1932; Gruenwald, 1973; Merker *et al.*, 1987; C.A. Smith and Moore, 1990).

These peculiar perforated maternal blood channels may indicate one possible route from the rodent-style narrow-meshed labyrinthine haemochorial placenta with a few massive maternal arterial channels supplying a subchorial lake directly to the widely spaced villous haemochorial placenta of man and the Old World monkeys with maternal blood supplied only from the basal plate (Figure 4.72).

Yet again, as many times before in this review, the variety of structures evolved by the different groups far outstrips our ingenuity in justifying them in terms of placental function.

Haemochorial placentation: number and form of interhaemal layers

All placentas so far examined seem to possess at least one persistent continuous trophectodermal layer as well as fetal endothelium to

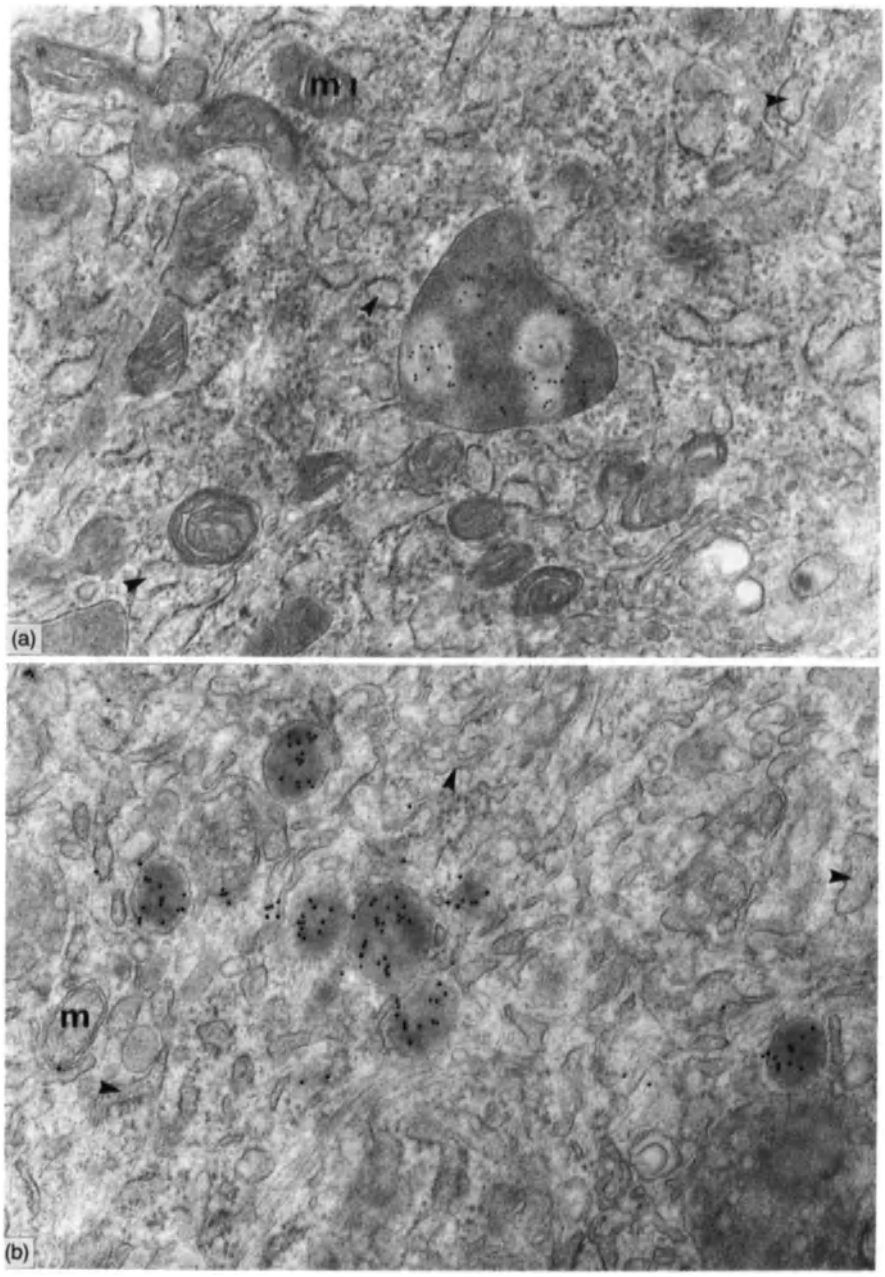


Figure 4.91 Haemochorial placenta of man. Micrographs of syncytiotrophoblast showing localization of hormone storage with immunogold techniques. (a) hCG is found in large irregular-shaped membrane-bound granules. (b) hPL in smaller more uniform-sized granules. Material fixed in glutaraldehyde and osmium and araldite embedded. m, mitochondria; arrowheads, rough endoplasmic reticulum. (a) First trimester placenta, × 28 000. (b) Term placenta, × 39 000.

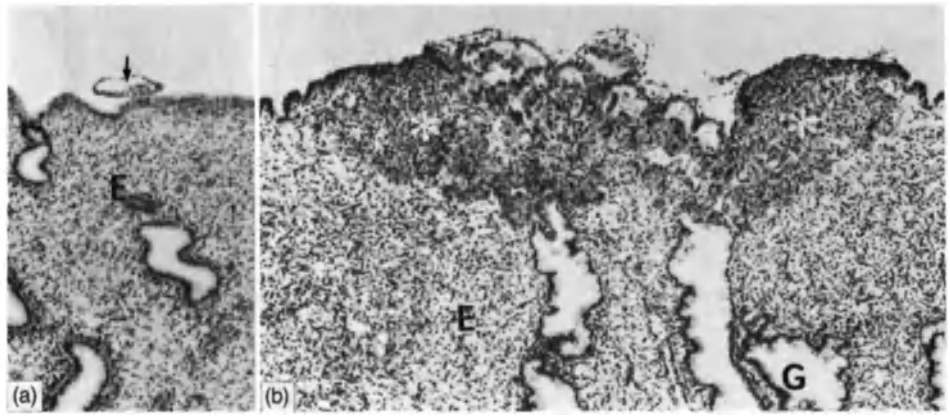


Figure 4.92 Haemochorial placental development in rhesus monkeys. Figures 4.92, 4.93 and 4.94 are at the same magnification. The major differences with human are the superficial implantation (arrow, conceptus) and development of epithelial plaque (asterisks in b and Figure 4.93a) by local proliferation of the uterine epithelium. The superficial endometrial blood sinusoids connect to the spaces within the syncytiotrophoblast (at the open arrows). Once the fetal cytotrophoblast and mesoderm have formed the initial broad villi (b and Figure 4.94a), their subsequent growth is by lengthening and subdivision (Figure 4.94a,b and c). There is little evidence for any significant further invasion of the uterine epithelium. At an equivalent stage of villus development the human (Figure 4.84a) and monkey (Figure 4.94a) placental structure is very similar, but there is no significant decidual development in the monkey endometrium (E). The cytotrophoblastic shell (X) which proliferates from the tips of the villi is particularly well demonstrated. C, chorionic plate; G, endometrial gland. (a) 9 dpc, × 50. (b) 12 dpc, × 50. (From Wislocki and Streeter, 1938.)

separate fetal and maternal circulations. The most efficient arrangement for maternofetal transport is for the maternal blood to circulate against the thinnest trophectodermal barrier compatible with immunological and other constraints. The most complete barrier is a syncytial sheet since there are no paracellular, non-selective routes possible: all molecules reaching the fetus can be screened for suitability by the trophectoderm. A haemomonosyncytiochorial structure of this sort is found in many different orders (section 4.3.6d), but despite its apparent advantages there exist also a remarkable variety of haemochorial trophectodermal organizations with one, two or three layers, each of which may be syncytial or cellular.

There is no evidence that nuclei in any vertebrate syncytium can divide. Recent evidence from human (Simpson *et al.*, 1992) indicates that the ratio of cytotrophoblast to syncytiotrophoblast is constant throughout gestation. Cytotrophoblast division followed by fusion into the trophoblast syncytium is the normal means of increasing its bulk and therefore its potential area. Since a syncytium has considerable plasticity the cytotrophoblast inputs can be scattered, and no continuous cellular layer is necessary. However, some such input is essential if the fetomaternal exchange surface is to enlarge continuously to the enormous extent reported (Baur, 1977) to support the fetal weight increase. In man the syncytiotrophoblast is produced initially from a continuous cytotrophoblast layer. This soon becomes discontinuous and the syncytium can differentiate locally to form the very thin vasculosyncytial membrane over fetal capillaries for example. Individual cytotrophoblast cells persist and are still capable of cell division even after the placenta is shed at term.

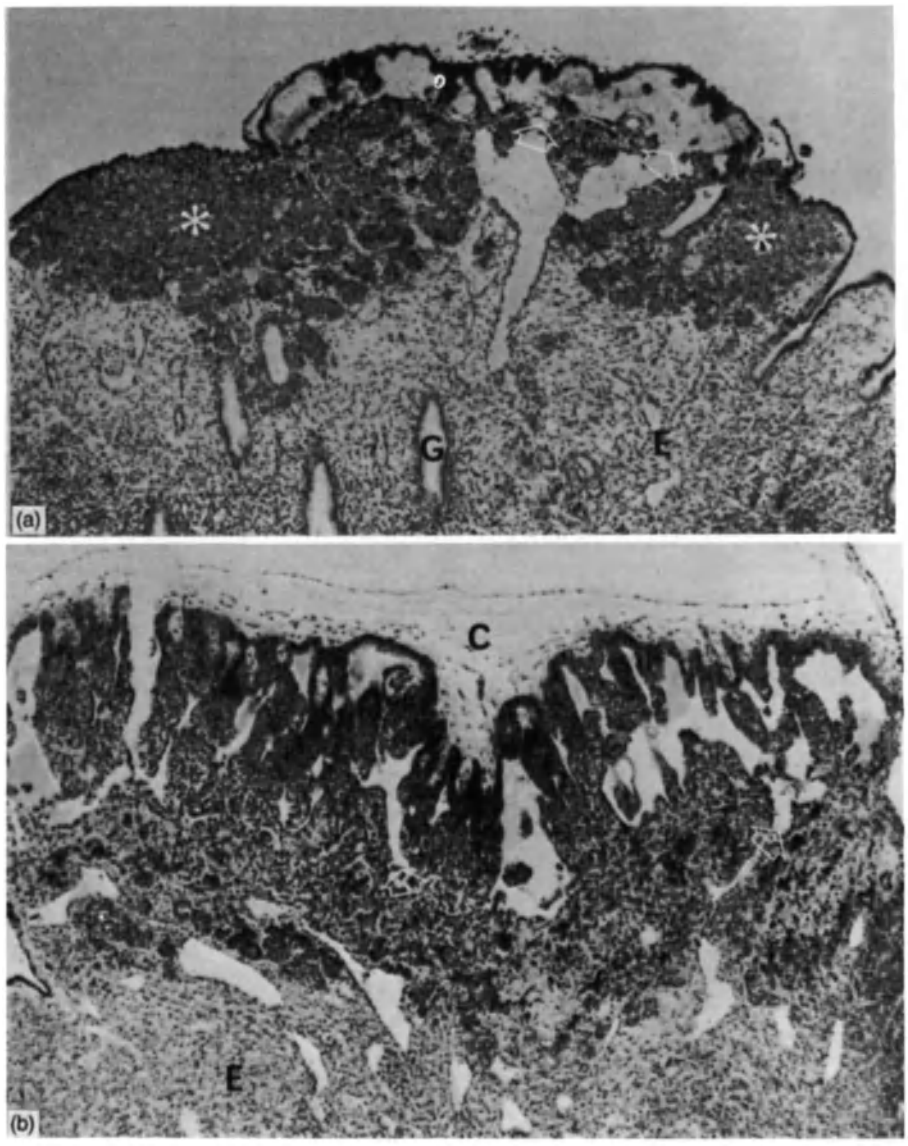


Figure 4.93 Haemochorial placental development in rhesus monkeys. For details see legend to Figure 4.92. (a) 14 dpc, × 50. (b) 17 dpc, × 50. (From Wislocki and Streeter, 1938.)

The characteristic mono-, di-, or trihaemochorial structure is established soon after implantation and, although the layers become thinner and the fetal capillaries indent the layers more deeply as pregnancy proceeds, there is no evidence for any structural simplification (tri- to di- to monochorial or the reverse) during development of any definitive placenta. At present little is known about the reasons for the many different patterns of layering found in haemochorial placentas. The more layers there are the more complete the spatial separation of functions can be. In a haemomonochorial placenta like that of man, numerous different proteins including hormones are synthesized and secreted independently (see Figure 4.91) but all use the same subcellular machinery (Patillo *et al.*, 1983; Morrish *et al.*, 1987). This must require a very complex hierarchy of controls which could be considerably simplified if several different types of cells are available, and there is good evidence for very different ultrastructure in the different layers of the same placenta (B.F. King and Hastings, 1977). Three layers obviously form a more complete barrier to invasion by maternal cells than one, and it is notable that haemomonochorial placentas always have a substantial fetal endothelial cell layer, whereas haemotrichorial placentas frequently show fenestrated fetal capillaries. The reason for the variety of haemochorial structures seems likely to lie in the multiplicity of functions served by the trophectoderm. With our present limited information we can only record the range and wonder.

Haemomonochorial

Cellular trophoblast: *Jaculus*

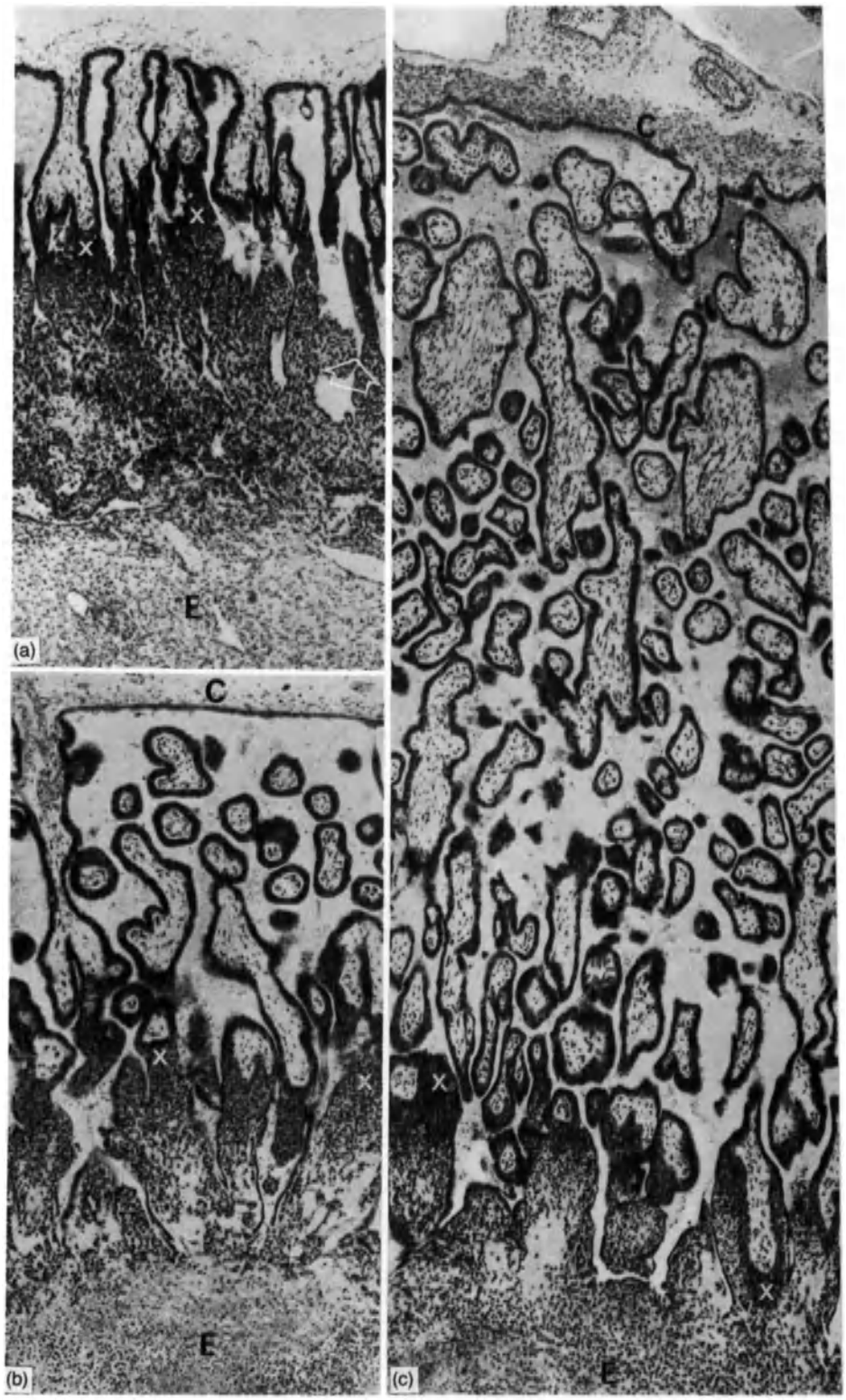
Myomorph rodents *Jaculus* (desert jerboa) and *Zapus* (jumping mouse) At implantation the germinative cytotrophoblast (layer III) produces a layer of syncytiotrophoblast (layer II), but both are very soon overgrown and disrupted by giant cells (layer I). These giant cells form the channels of the labyrinth in which the maternal blood circulates (Figures 4.76, 4.96 and 4.97).

They are derived from cytotrophoblast cells at the edge of the placental disc (B.F. King and Mossman, 1974; Mossman, 1987).

Hyracoidea, Procavia (rock hyrax) This order is thought to be related to the elephants on skeletal evidence, but the placental structures are very different since the Elephantidae are endotheliochorial (section 4.3.6c). The hyrax cytotrophoblast produces a thick layer of cells replacing the upper layers of the endometrium in the implantation chamber (Sturgess, 1948). By the time the fetus has reached a crown–rump (CR) length of 12 mm (midgestation; Wislocki and Westhuysen, 1940) this cellular layer is almost as thick as is the definitive placenta near term (50 mm CR). The cellular layer transforms into a trabecular meshwork forming channels with cellular walls (Oduor-Okelo *et al.*, 1983) through which the maternal blood drains back to the uterine veins (Figure 4.96).

The blood is delivered to the fetal side of the placenta by persistent maternal arterial channels which pass right through the fetal cell layer. The fetal allantoic arterioles invade the cytotrophoblastic trabeculae as they develop, delivering fetal blood to the maternal side of the placenta, from whence it drains back through a capillary meshwork establishing a grossly countercurrent system of fetal and maternal blood flows. This developmental pattern is very similar to that described (section 4.3.6d) for the New World monkeys but they are haemomonosyncytiochorial.

Bats, Vespertilionidae and Molossidae These orders have genera (*Miniopteris*, Kimura and Uchida, 1984; *Tadarida*, Stephens, 1962, 1969) which produce chorioallantoic definitive placentas based solely on cytotrophoblastic proliferations which engulf the maternal vascular channels. This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produces a labyrinth lined only with cellular cytotrophoblast (Figure 4.11b). Both genera also have transient accessory endotheliodichorial placentas (cytotrophoblast plus syncytiotrophoblast (Stephens and Cabral, 1971, 1972).

Syncytiotrophoblast A wide variety of orders have members with haemomonosyncytiochorial membranes in their definitive placentas: all anthropoids (Figures 4.87, 4.98); one carnivore, hyena (Figure 4.10a) (Oduor-Okelo and Neaves, 1982); two insectivores, cane rat and elephant shrew (Oduor-Okelo, 1984a,b); several rodents such as Marmots (Figure 4.10b), chipmunk, squirrels (Mossman, 1987), guinea pig (Figures 4.6 and 4.7) (Kaufmann, 1981b) and chinchilla (B.F. King and Tibbits, 1976); armadillos (Dasypodidae; Enders, 1982) and anteaters (Myrmecophagidae; Becher, 1931; Walls, 1938); and several bats (section 4.3.6c).

Although nominally haemo**mono**chorial, in practice there are probably always cytotrophoblast cells scattered over the fetal side of the syncytiotrophoblast (Figure 4.98). These are necessary to support the vast increase in area by continual division and fusion into the syncytium, because there is no evidence that nuclei in vertebrate syncytia can divide.

Haemodichorial

There are two types:

1. The cytotrophoblast which generates the syncytiotrophoblast persists as a continuous layer. The circulations are separated by fetal endothelium, cytotrophoblast and syncytiotrophoblast.

This is characteristic of several orders of bats for example, Thyropteridae (Wimsatt and Enders, 1980) (Figure 4.99), Vespertilionidae (Enders, 1982) and Desmodontidae (Bjorkman and Wimsatt, 1968).

All the bats show a characteristic intrasyncytial lamina close to the apex (Figure 4.99).

The rabbit has a similar dichorial arrangement but the cellular layer is very different in structure from the rather uniform columnar cellularity of the bats. (Figures 4.81 and 4.98; Enders, 1965; Samuel *et al.*, 1975). It may be that both rabbit layers are independently generated by cellular inserts on the haemotrichorial pattern (section 4.2.7a).

2. The generative cytotrophoblast is discontinuous but there is an additional cellular layer between the syncytiotrophoblast it generates and the maternal blood. This pattern has been described only in two rodents, the beaver (Fischer, 1971) and the springhaas *Pedetes* (Fischer and Mossman, 1969). There is a considerable initial cytotrophoblast proliferation at the placental site. The syncytiotrophoblast-bound fetal villi grow into this layer, producing a definitive placenta with a cellular outer layer bathed in maternal blood underlain by a syncytial layer. The details of the development of the two layers are not clear.

Haemotrichorial

This is found only in myomorph rodents with just a single pattern of layering in the cricetids (Figures 4.100 and 4.101) (gerbil) and murids (rats, mice, hamsters) (Enders, 1965; B.F. King and Hastings, 1977).

The circulations are separated by fetal endothelium, two syncytial layers and an outer fenestrated cellular layer (section 4.3.6d).

Figure 4.94 Haemochorial placental development in rhesus monkeys. For details see legend to Figure 4.92. (a) 20 dpc, × 50. (b) 29 dpc, × 50. (c) 35 dpc, × 50. (From Wislocki and Streeter, 1938.)

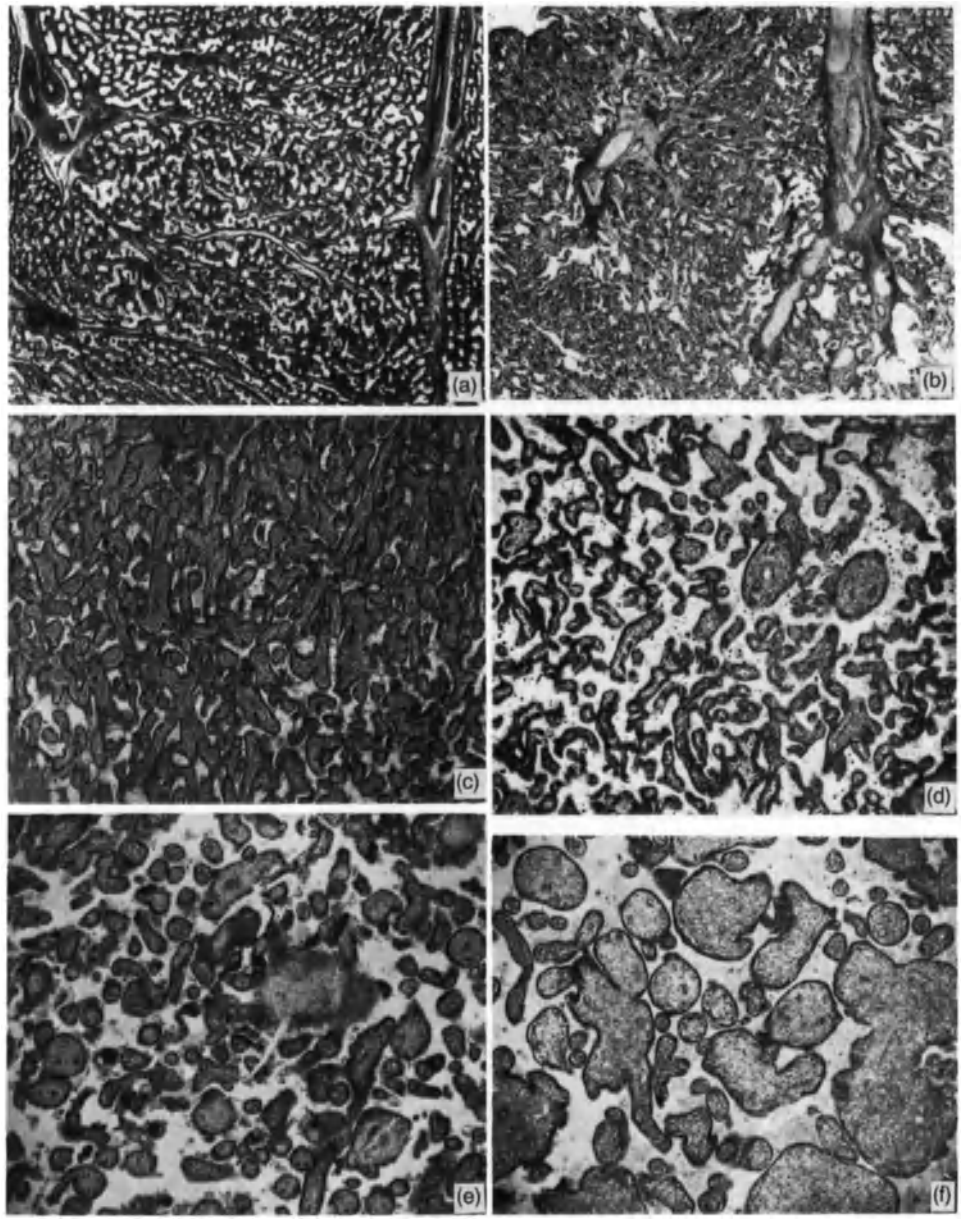

Figure 4.95 Haemochorial placentation. Comparison of the size of the fetal trabeculae or villi in 'mature placentae at approximately equivalent stages'. Description and plate from Wislocki (1929). (a) *Cynocephalus* (*Galaeopithecus*), the flying lemur, a dermopteran probably most closely related to the insectivores. The placenta consists of narrow interconnected trabeculae very similar to that of the New World monkey *Ateles*, shown in (b) at identical magnification. V, major fetal villi carrying blood directly to the maternal side. (c–f) All at the same magnification to show the range of placental architecture. (c) The New World monkey *Alouatta* has a narrow trabeculate placenta. (d) The Old World *Colobus* monkey shows a mixture of villi and narrow trabeculae. (e) The Old World gibbon has larger villi with frequent syncytial connections. (f) The anthropoid gorilla shows a completely free villous structure, as in *Homo*. (a) *Cyncocephalus*, 84 mm crown–rump length, × 14. (b) *Ateles*, 187 mm CR, × 14. (c) *Alouatta*, 130 mm CR, × 42. (d) *Colobus*, 143 mm CR, × 42. (e) *Hylobates*, 102 mm CR, × 42. (f) *Gorilla*, 89 mm CR, × 42. (From Wislocki, 1929.)

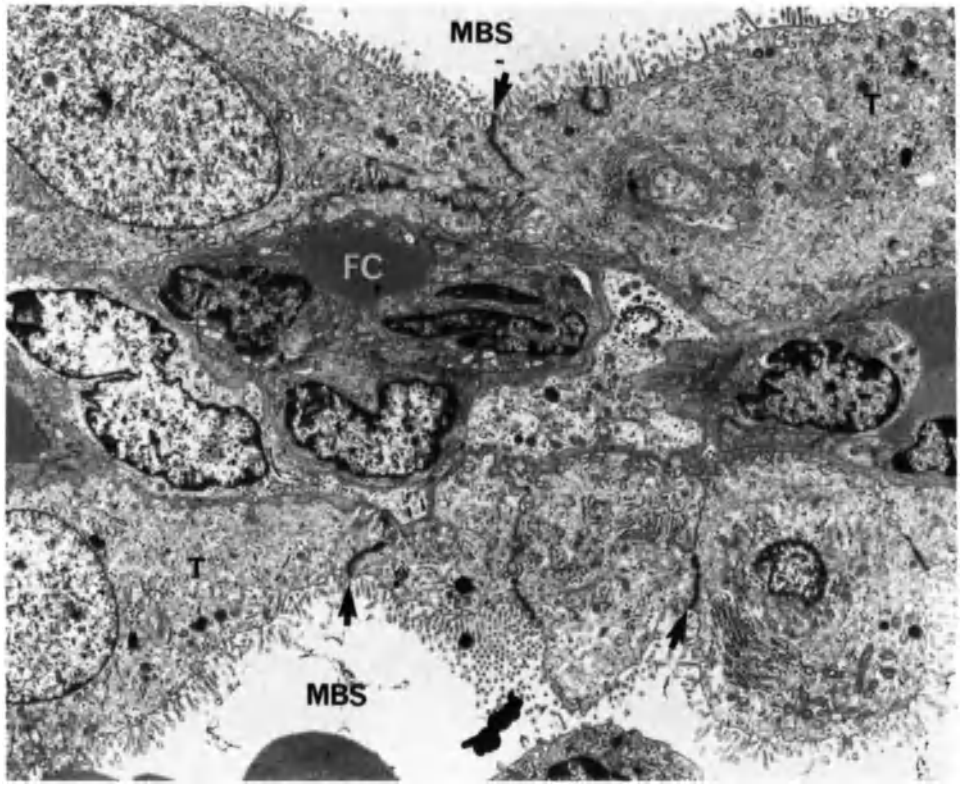

Figure 4.96 Haemochorial placentation. Monochorial cellular trophectoderm in hyrax. The interhaemal membrane is clearly cellular in hyrax with apical tight junctions (arrows) obvious in this mid-term placenta. FC, fetal capillary; MBS, maternal blood space; T, trophectoderm. Mid-pregnancy, × 6000.

4.4 PLACENTAL ENDOCRINE FUNCTION

4.4.1 PLACENTAL HORMONES

The endocrinology of pregnancy is dealt with in detail in Chapter 11, this volume; however, a description of placentation would be incomplete without some consideration of the general principles of placental endocrine function, and the discussion that follows is limited to these aspects.

As indicated above, endocrine mechanisms have been utilized by a wide range of species to solve many of the problems associated with intrauterine gestation (Fisher, 1986). Hormones secreted by the placenta have been shown in different species to block further ovarian follicular development and ovulation (progesterone and possibly inhibin), thereby preventing superfetation; to block the contractile response of the uterus to a foreign body (such as an embryo) placed in it (progesterone, relaxin); to ensure the continued secretion of nutrients by the maternal endometrial glands (progesterone); to mobilize metabolic reserves such as fats within the maternal organism and to cause mammary growth and development (placental lactogens) so that milk secretion ensues post partum. In some species at the end of pregnancy, the placenta is involved in signalling (through changes in secretion of progesterone and oestrogens) to the mother that the fetus has developed sufficiently for birth to occur and to cause the mother to demonstrate characteristic maternal behaviour

patterns such as nest building. It is perhaps not surprising that endocrine mechanisms should be so important in communication between the fetus and mother, since there is no nervous connection between the two organisms.

(a) Steroids

Progesterone

Progesterone has been shown to be produced by every placenta in which it has been examined in detail. The most frequently applied method for detecting placental progesterone secretion is measurement in peripheral plasma by radioimmunoassay or competitive protein binding; examples of data obtained in this way are illustrated in Figure 4.102.

This method clearly fails to distinguish between progesterone secreted by the placenta and that secreted by other organs, most notably the corpus luteum. Since progesterone is required for the maintenance of gestation in most species, the production of progesterone by the placenta can be detected by eliminating other sources, by ovariectomizing or lutectomizing animals at various stages of gestation, and determining whether pregnancy is maintained. By this means it can be shown, for example, that the sheep's placenta produces sufficient progesterone to maintain gestation in the absence of the ovaries after day 50 post coitum (Casida and

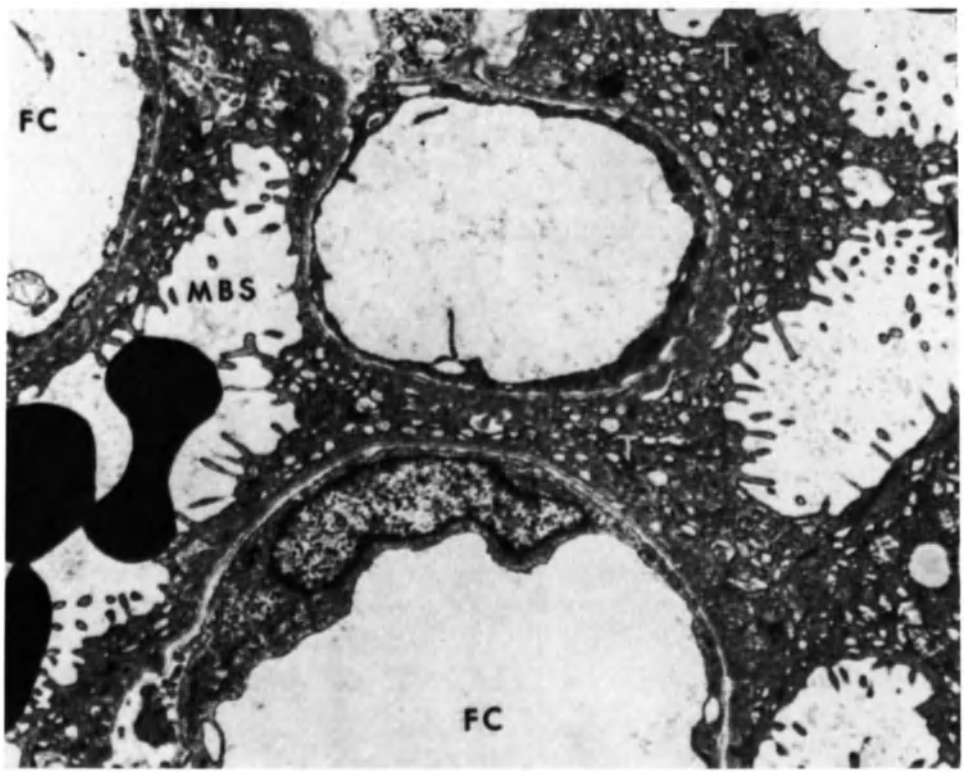

Figure 4.97 Haemochorial placentation. In the monochorial cellular trophectoderm of the rodent *Zapus* it is not possible to distinguish the cellular boundaries at this magnification, the trophectoderm cells are very extensive and very attenuated. FC, fetal capillary; MBS, maternal blood space; T, trophectoderm. Late pregnancy, × 8000. (From King and Mossman, 1974.)

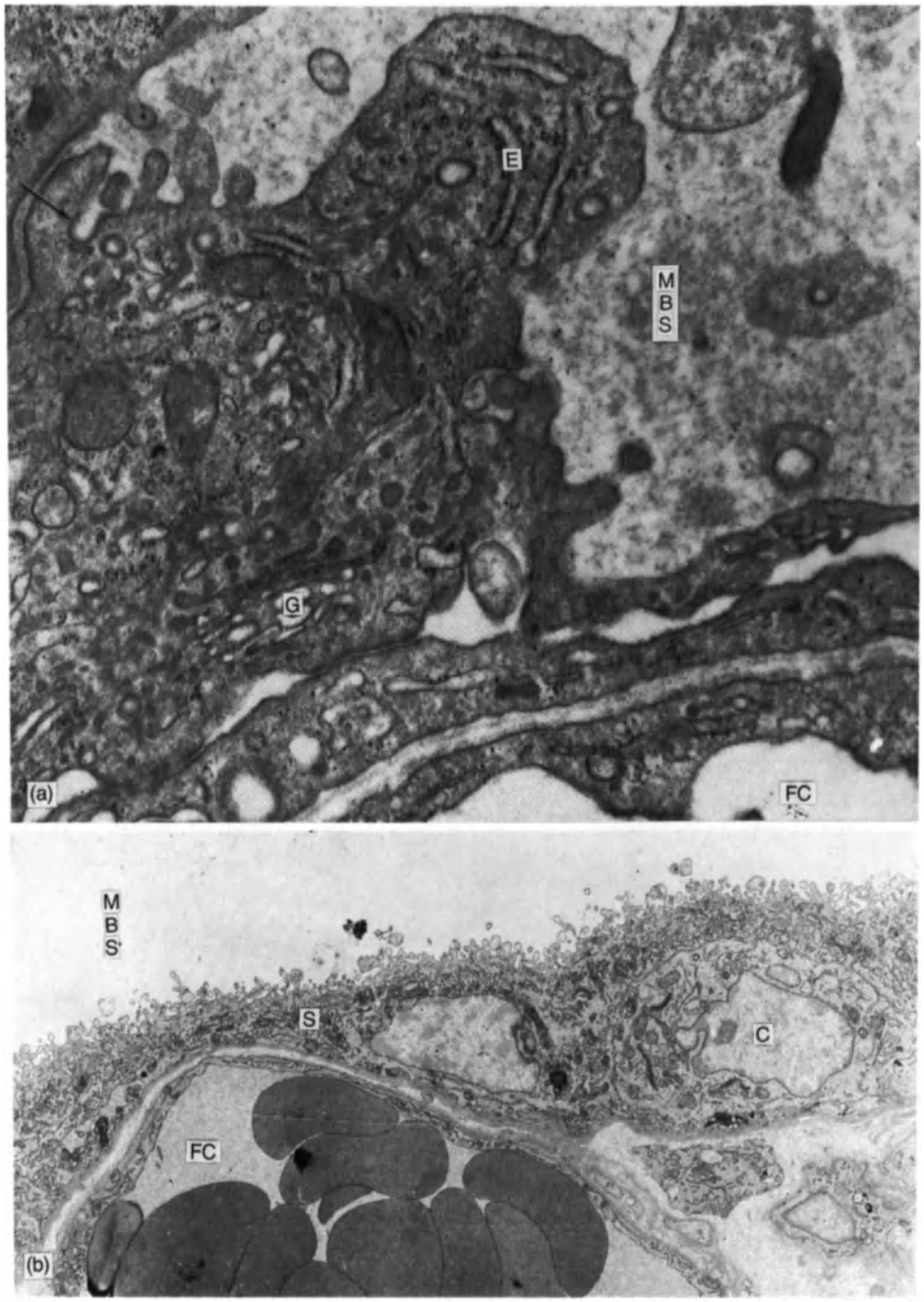

Figure 4.98 Haemochorial placentation. (a) Interhaemal membranes in the haemodichorial rabbit placental labyrinth. The trophoblast layer in contact with maternal blood (MBS) shows numerous coated pits (arrow), abundant rough endoplasmic reticulum (E) and a Golgi body (G). The inner trophoblast layer shows no membrane specializations. FC, fetal capillary. (a) Late pregnancy, × 28 500. (From Enders, 1965.) (b) The human, nominally haemomonochorial, syncytiotrophoblast (S) contains cytotrophoblast cells (C) capable of division and differentiation into syncytium right up to the time of parturition. Term placenta, × 4400.

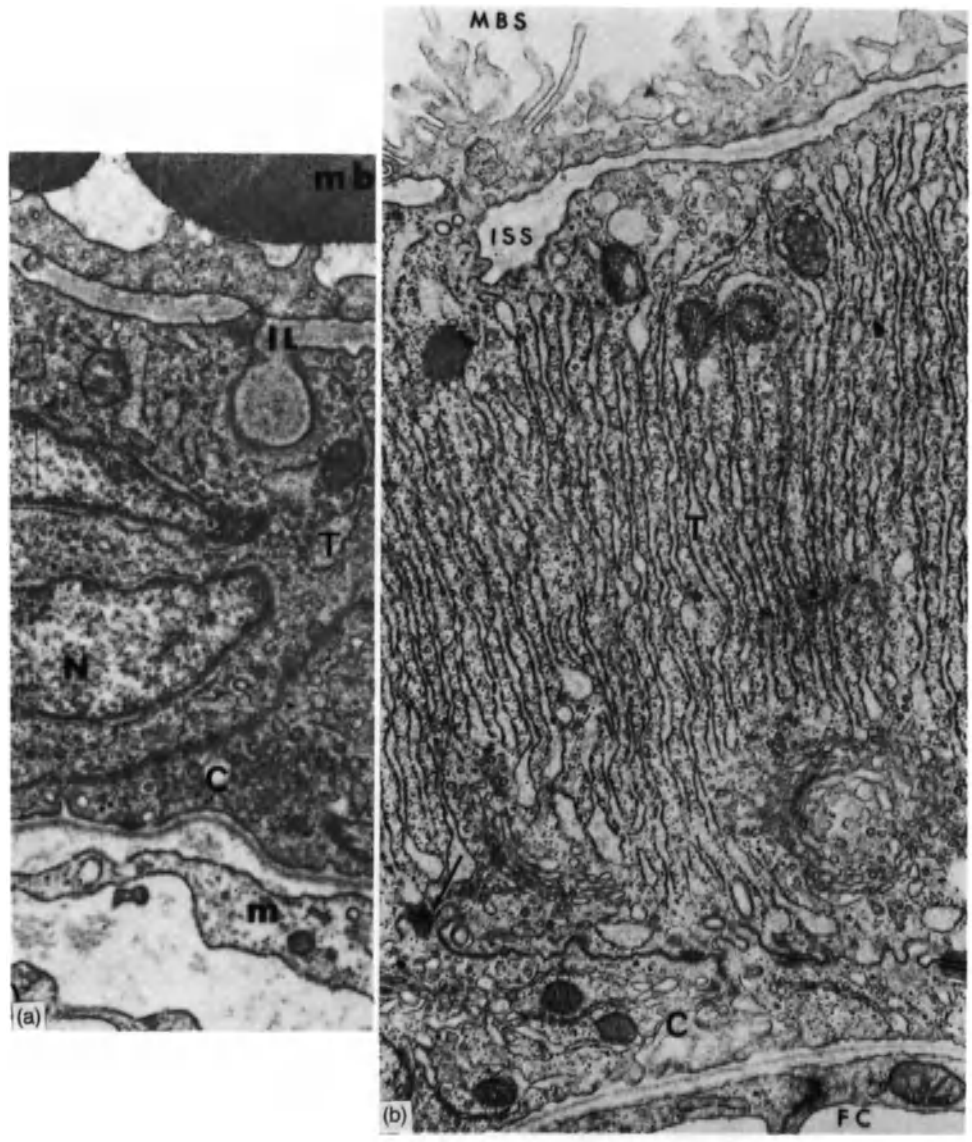

Figure 4.99 Haemodichorial bat placentae. The intrasyncytial lamella (IL in a, ISS in b) in the apical syncytiotrophoblast (T) is much more uniform in thickness, texture and separation from the maternal blood (mb in a, MBS in b) than those shown in Figures 4.10 and 4.11. Compare the structure with that of the endotheliochorial placenta (cat) in Figure 4.68, which is said to be characteristic of many other genera of bats. C, cytotrophoblast; FC, fetal capillary; N, nucleus; M, mesoderm cell. (a) *Thyroptera* near-term placenta, × 17 500. (From Wimsatt and Enders, 1980.) (b) *Myotis* near term placenta, × 22 000. (From Enders, 1982.)

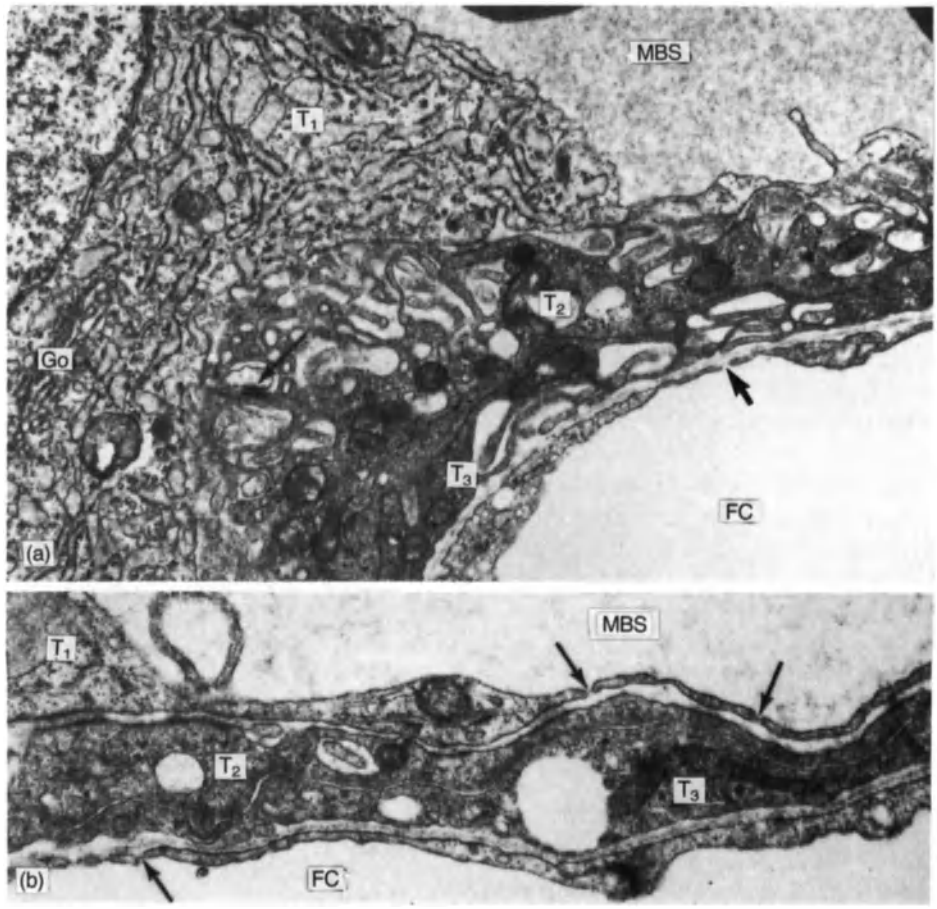

Figure 4.100 Haemotrichorial placentation in myomorph rodents. The triple layering is remarkably consistent in Muridae (rats, mice) and these examples of Cricetidae. Bounding the maternal blood space (MBS) layer T1 is usually cellular but very irregular with thin, discontinuous areas (b, arrows) next to regions containing the nucleus and abundant cytoplasm (a) with a lot of rough endoplasmic reticulum and Golgi bodies (a, Go). Layer T2 is syncytial, connected to T1 via the occasional desmosome (a, arrow), and is usually the widest, frequently showing considerable elaboration of the apical plasmelemma. (a and c) T3 is thinnest and most uniform, linked to T2 via desmosomes and gap junctions and occasionally having proliferations of the basal plasmalemma (a) adjacent to the fenestrated (a and b; upward arrows) fetal capillaries (FC). M, mitochondrion. Arrow in (c), cell boundary junction in T1. (a) *Peromyscus*, near-term placenta, × 17 500. (b) *Lemmus*, near-term placenta, × 22 600. (From B.F. King and Hastings, 1977.)

Warwick, 1945; Denamur and Martinet, 1955). The stage of gestation at which the placenta first produces sufficient progesterone to maintain pregnancy in the absence of the corpus luteum has been termed the stage of the 'luteoplacental shift' (see Csapo, 1977). However, there are animals in which the placenta produces progesterone, but in quantities which are insufficient to maintain gestation following ovariectomy. The goat and the pig are examples of such species; in both cases, ovariectomy at any stage of gestation leads to pregnancy loss (Drummond-Robinson and Asdell, 1926; Meites, *et al.*, 1951; Du Mesnil Du Buisson and Dauzier, 1957; Cowie *et al.*, 1963; First and Staigmiller, 1973). In these animals, as well as in sheep, placental progesterone secretion can be detected by replacing the luteal progesterone in ovariectomized individuals with a synthetic progestagen that does not interfere in the progesterone assay, and measuring progesterone in the maternal peripheral, uterine venous or fetal vasculature. This procedure shows that in goats the placenta secretes increased quantities of progesterone between days 120 and 140 (Sheldrick, Ricketts and Flint, 1980; Figure 4.103), and in pigs it has been used to demonstrate placental progesterone secretion after day 103 (V.A. Craig and A.P.F. Flint, unpublished observations; Figure 4.104).

In sheep, Ricketts and Flint (1980) demonstrated that placental progesterone secretion began at between 50 and 70 dpc, thereby confirming the information obtained after ovariectomy without replacement therapy. This information also confirms the conclusion reached by Thorburn and Mattner (1971) from measurements of progesterone in the sheep subcutaneous mammary vein, which had been anastomosed to a utero-ovarian vein in order to facilitate collection of uterine venous blood by percutaneous venepuncture.

In animals in which the placenta secretes little progesterone it may produce a related but biologically inactive steroid. The goat's

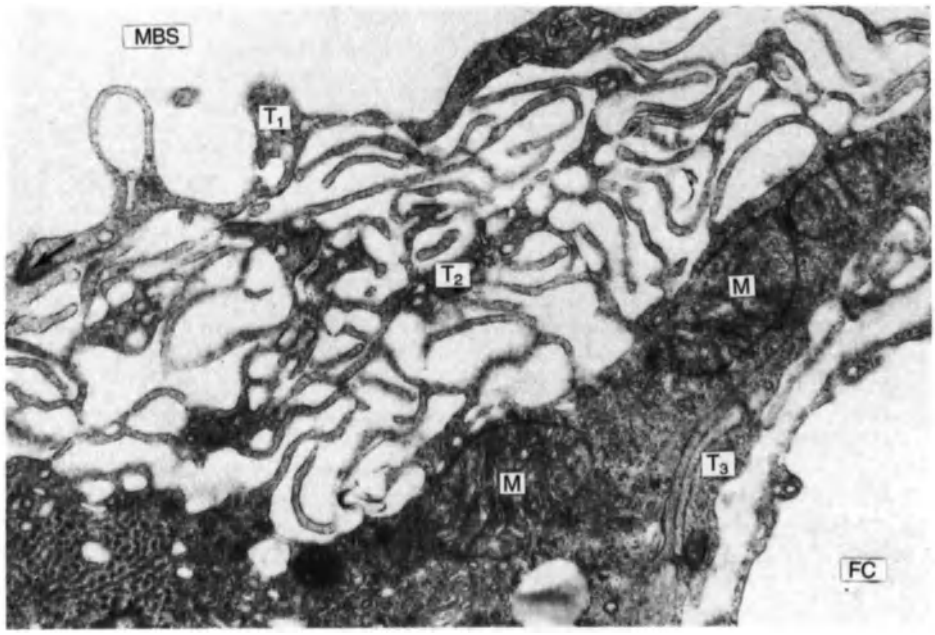

Figure 4.101 Haemotrichorial placentation in myomorph rodents. *Cleithryonomys*, near-term placenta, × 21 300. For details see legend to Figure 4.100. (From B.F. King and Hastings, 1977.)

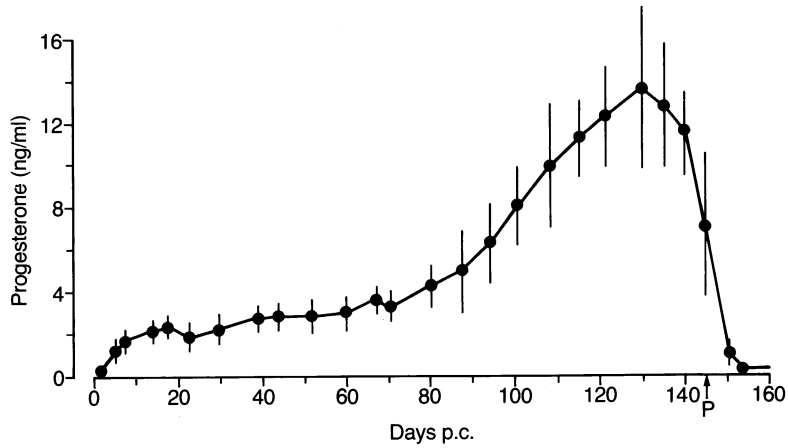

Figure 4.102 Concentrations of progesterone in peripheral circulation in sheep during pregnancy. Note that the corpora lutea are unnecessary for pregnancy maintenance after day 50, and that progesterone concentrations fall before parturition (P) at term. (After Bassett *et al.*, 1969, redrawn.)

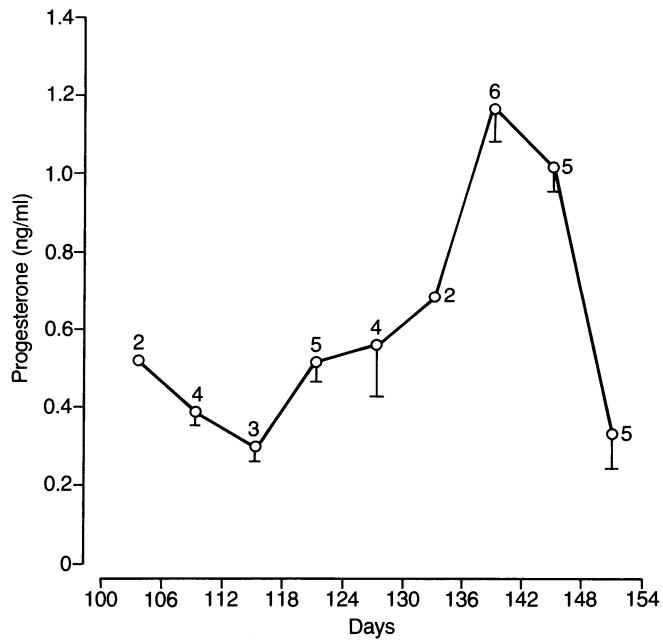

Figure 4.103 Concentrations of progesterone in peripheral maternal plasma during the last third of gestation in four ovariectomized or lutectomized goats. The number of samples assayed is given at each point. All samples were obtained before parturition. Pregnancy was maintained following removal of the corpora lutea by administration of medroxyprogesterone acetate. All kids were born alive. (After Sheldrick *et al.*, 1980, redrawn.)

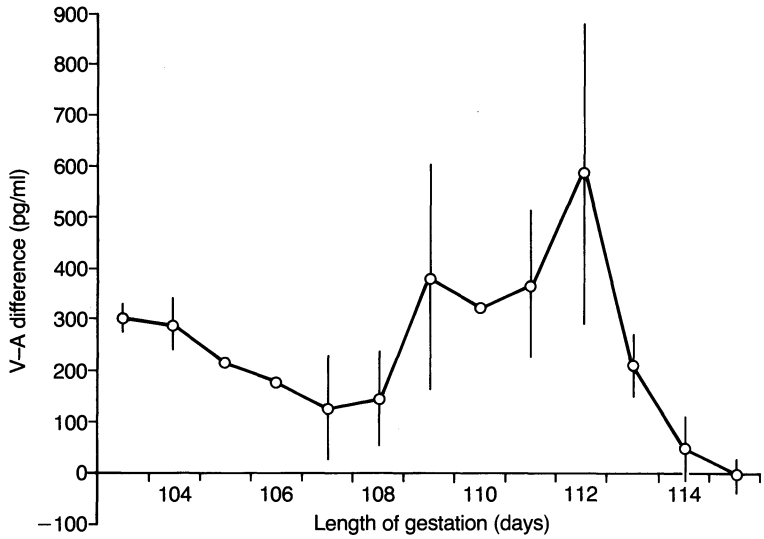

Figure 4.104 Venoarterial concentration differences for progesterone across the uterus in ovariectomized pregnant gilts treated with medroxyprogesterone. Values represent carotid arterial concentrations subtracted from uterine venous concentrations (four animals); mean venous concentration between days 103 and 112 was approximately 1000 pg/ml. Ovariectomy was performed between days 93 and 102; survival of the piglets was normal. (After V.A. Craig and A.P.F. Flint, unpublished.)

placenta synthesizes progesterone in large quantities, but reduces it to 5β-pregnane-3α, 20α-diol before secreting it (Flint *et al.*, 1978); radioimmunoassay of this latter steroid in peripheral plasma during gestation shows that levels change in a manner similar to those of progesterone in sheep (Figure 4.105; Sheldrick, *et al.*, 1981).

One possible advantage resulting from this characteristic is that the supply of precursors for oestrogen synthesis is reduced; oestrogens are luteolytic in the goat and therefore cause pregnancy loss. In those species in which the placenta produces large quantities of progesterone (e.g. the sheep and man) the corpus luteum regresses, presumably due to reduced circulating levels of luteinizing hormone caused by high circulating progesterone levels (see Sheldrick *et al.*, 1980).

Oestrogens

As is the case with progesterone, oestrogen secretion is a common placental endocrine function. However, there are marked differences between species in the metabolic pathways leading to placental oestrogen synthesis. Some animals, notably ruminants, produce oestrogens by synthesis from C_{21} steroid precursors (pregnenolone and progesterone); others (e.g. man), lacking the placental 17α-hydroxylase and C-17,20-lyase necessary for this pathway, produce oestrogens by aromatization of androgens secreted by the fetal adrenal glands, primarily dehydroepiandrosterone sulphate. These latter are said to possess a 'fetoplacental unit', because both compartments are required to complete the steroidogenic pathway to oestrogens. The only species so far thought to possess a fetoplacental unit are man, the equids and, possibly, seals. Examples of patterns of oestrogen synthesis through pregnancy are illustrated in Figures 4.37 (horse) and 4.106 (man).

Oestradiol-17β, generally regarded as the most potent oestrogen on administration in bioassays, is not always the major circulating oestrogen from a quantitative point of view.

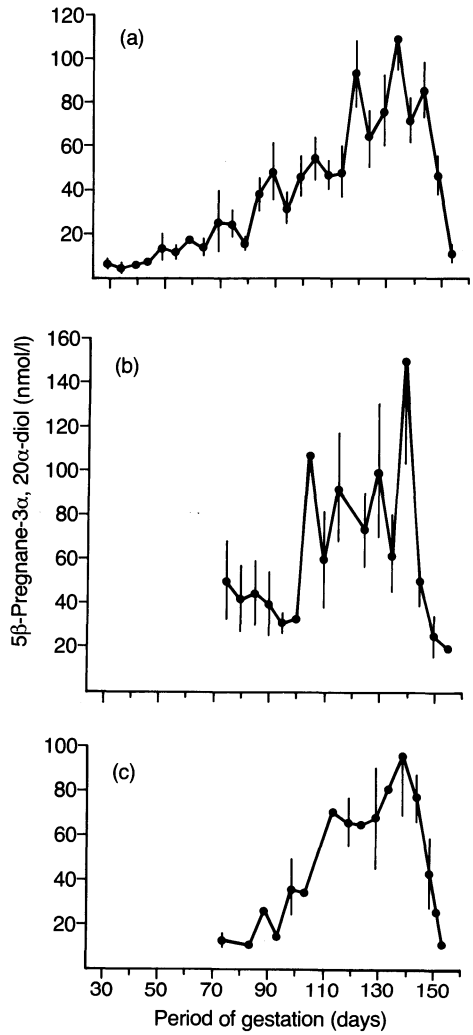

Figure 4.105 Circulating concentrations of 5β-pregnane-3α, 20α-diol in goats during pregnancy. Levels were measured in jugular venous plasma by specific radioimmunoassay from (a) eight untreated animals; (b) five animals treated with medroxyprogesterone acetate and (c) three lutectomized, medroxyprogesterone acetate-treated animals. All samples were taken before delivery of the kids. Lutectomy was performed between days 71 and 75. All kids were born alive. (After Sheldrick *et al.*, 1981, redrawn.)

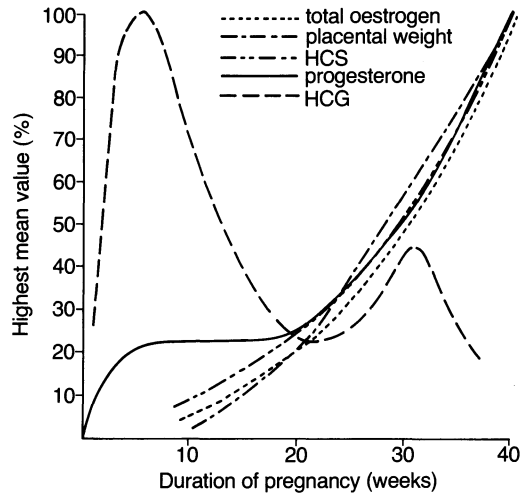

Figure 4.106 Diagram representing changes in placental weight and peripheral plasma concentrations of placental hormones during human pregnancy. Values are normalized to the highest level reached (= 100%).

In man the oestrogen circulating in highest concentration is oestriol, and in goats oestradiol-17α. In most species conjugated oestrogens (sulphates and glucuronides) circulate in higher concentrations than unconjugated oestrogens, though this may reflect more complete hepatic clearance of the latter rather than greater placental secretion of the former.

Control of placental steroid secretion

Factors controlling placental steroid secretion are complex. In some instances the quantity of a steroid produced is proportional to the weight of the placenta; in others the rate of steroid production is limited by supply to the placenta of a substrate; and in yet others there are changes in the activities of placental enzymes at defined stages of gestation which play a major role in influencing the pattern of steroids produced.

An example of the first of these mechanisms is the way in which maternal circulating progesterone concentrations change throughout pregnancy in man (Figure 4.106);

the circulating level clearly follows closely the changing mass of the placenta for the greater part of pregnancy, and although the actual concentration of progesterone in plasma is determined largely by factors influencing its clearance rate (for example, proteins such as cortisol-binding globulin, which also avidly binds progesterone), the increase during gestation is principally due to increasing placental weight.

Increasing placental weight would appear also to contribute to the increasing concentration of oestrogen in the maternal circulation during human pregnancy (Figure 4.106); however, circulating oestrogen levels are limited by the supply to the placenta of fetal adrenal androgens, and can be increased by administering such compounds (e.g. dehydroepiandrosterone sulphate) to the mother. The increase in oestrogen level with advancing pregnancy reflects increased fetal adrenal size. A similar mechanism is thought to operate to control placental production of the B-ring unsaturated oestrogen, equilin, in the horse and other equids; in these species equilin production occurs transiently in mid-gestation and is thought to result from the production of a precursor by the fetal gonad, which hypertrophies at this stage (Figure 4.37) (Pashen *et al.*, 1983). The identity of the precursor is not established, although it has been suggested that it arises via a novel steroid biosynthetic pathway (the sesquiterpene pathway; Bhavnani and Woolever, 1978; Tait *et al.*, 1982).

The third form of control of placental endocrine function, through changes in placental enzyme activity, is neatly exemplified by the effect of raised fetal cortisol concentrations on progesterone and oestrogen secretion at term in ruminants. At term in the sheep the rapid rise in fetal circulating concentrations of cortisol results in the activation of the placental enzyme 17α-hydroxylase/C-17,20 lyase. This converts progesterone into oestrogens, resulting in increased secretion of mostly oestrone sulphate (A.B.M. Anderson *et al.*, 1975; Steele *et al.*, 1976; see also Flint, 1983). These changes in maternal steroid concentrations are thought to cause the onset of uterine contractions by inducing prostaglandin synthesis in the uterus (Mitchell and Flint, 1978b; Thorburn, 1991). The effect of cortisol on the placenta is exerted directly; cortisol induces 17α-hydroxylase in isolated explants of sheep's placenta *in vitro* (Ricketts *et al.*, 1980), and the presence of the glucocorticoid receptor in the placenta is also consistent with a direct action (Flint and Burton, 1984). Similar enzymic changes occur in the goat's placenta at term, in response to raised fetal circulating levels of cortisol (Flint *et al.*, 1978), which are thought to account for the dramatic drop in maternal levels of 5β-pregnane-3α, 20α-diol before term as well as the rise in oestrogen (Sheldrick *et al.*, 1981).

(b) Polypeptide hormones

Polypeptide and smaller peptide hormones secreted by the placenta or acting locally in an autocrine manner have been identified in a limited number of species, principally in primates, rodents and the large domestic animals. The polypeptide hormones studied in depth fall into two groups, the chorionic gonadotrophins and placental lactogens; the former are related structurally and evolutionarily to the pituitary hormone LH and the latter to prolactin/growth hormone (for review see H.J. Stewart *et al.*, 1988).

Chorionic gonadotrophins

The existence of chorionic gonadotrophins with FSH- and LH-like activities has been recognized since observations of the stimulation of gonadal function in rodents by extracts of human placenta and urine over half a century ago (Aschheim and Zondek, 1927; for comprehensive recent reviews see Allen, 1975; Talamantes and Ogren, 1988; Boime *et al.*, 1989). It is however, only with the application of recombinant DNA techniques that

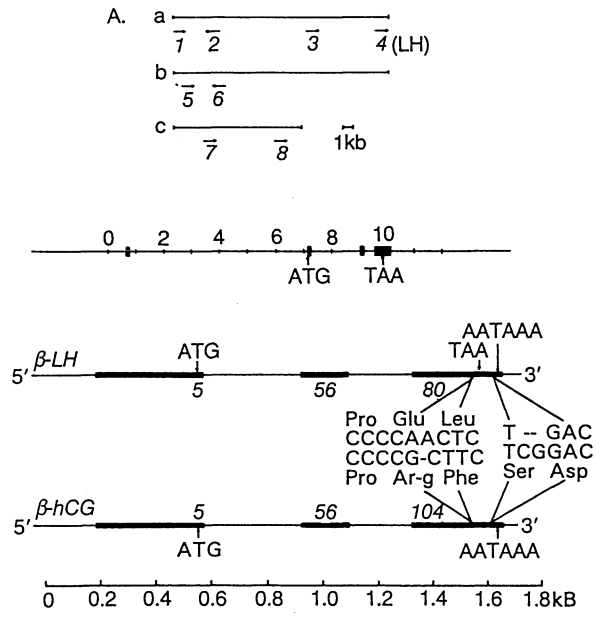

Figure 4.107 Structure of hCG and LH genes. (a) Organization of seven hCG-β and one LH-β subunit gene on three genomic fragments; arrows indicate direction of transcription. (b) Structure of human glycoprotein-α gene; figures above line indicate kilobases relative to an arbitrary point, and exons are thickened. ATG and TAA indicate translation start and stop sites respectively. (c) Evolution of hCG-β from LH-β; exons are thickened, scale is in kilobases and expanded regions illustrate deletions and insertions in the LH sequence giving rise to hCG, as well as the amino acids. Figures above and below exons indicate the number of amino acids coded for by each exon. (From H.J. Stewart *et al.*, 1988, redrawn.)

we are now beginning to understand the origin of these hormones. The chorionic gonadotrophins are glycosylated proteins of molecular weight 38 kDa which have been investigated most fully in primates and equids. They consist of two subunits, α and β. In man, the α-subunit is identical to the α-subunits of the pituitary glycoproteins, LH-α, TSH-α, FSH-α, there being only one gene for this protein which is expressed in the placenta as in the pituitary. In contrast the β-subunit of chorionic gonadotrophin differs from that of LH. It is closely homologous to, but is longer than, LH-β; it has evolved from LH-β through a frameshift mutation, leading to loss of the translation termination codon, with subsequent readthrough into the normally untranslated region of the LH-β gene (Figure 4.107).

As a result a further 24 amino acids are present at the C-terminal end of the hCG-β subunit that are absent from the LH-β chain. The termination codon (UAA) utilized by hCG-β is part of the polyadenylation sequence (AAUAA). There are six hCG-β (but only one LH-β) genes, all arranged in a 58-kbp region on chromosome 19; it is thought however that only two or possibly three are actively transcribed, the remainder representing pseudo-genes. The FSH-β, TSH-β and common α-subunit genes are mapped to chromosomes 11, 1 and 6 respectively; therefore it appears that the duplication of the ancestral β-subunit gene and separation of the resulting genes preceded evolution of hCG-β. Since many species (e.g. mice, Dracopoli *et al.*, 1986) lack a chorionic gonadotrophin, the evolution of the hCG-β gene

must post-date the evolutionary divergence of mice and the primates. A similar arrangement of CG-β genes occurs in the baboon (Crawford *et al.*, 1986); there are, however, considerable nucleotide sequence differences between hCG-β and the baboon hormone.

Chorionic gonadotrophins have not been studied in such detail in other primate species, and most of the evidence for their existence in primates is based on radioimmunoassays and bioassays. Using these procedures, chorionic gonadotrophins have been identified in the placentas of apes and of Old and New World monkeys (Hobson and Wide, 1972; Hodgen *et al.*, 1974). A chorionic gonadotrophin has been isolated from the guinea pig placenta (Bambra *et al.*, 1984), but the evidence for similar molecules in rat mouse or hamster placentas is at present only circumstantial (Talamantes and Ogren, 1988).

The phenomenon of gonadotrophin production by the equine conceptus has been studied in considerable depth; the ready availability of this highly gonadotrophic material (previously known as 'pregnant mare serum gonadotrophin', PMSG; more recently equine chorionic gonadotrophin, eCG) has produced an industry of considerable importance as a result of its use in stimulation of gonadal function, with applications in the treatment of infertility (including human infertility) and in the production of oocytes for embryo transfer. It was long considered that eCG differed from LH in a manner similar to that in which hCG differed from hLH; this appeared particularly likely in view of the fact that eCG, like hCG has a C-terminal extension relative to most LHs. However, it has now been shown that eCG and eLH have identical amino acid sequences; the only difference between them is in the way in which they are glycosylated (see H.J. Stewart *et al.*, 1988).

The observation that hCG is produced by trophoblast neoplasms in very large quantities provides a ready diagnostic method for following the growth and regression of choriocarcinomas and molar pregnancies (see Patillo *et al.*, 1983). The finding that either α-subunit or hCG itself is produced by a range of other non-trophoblastic neoplasms, including adenocarcinomas of the stomach, in which serum hCG can reach levels similar to those found in the first trimester of pregnancy, stimulated speculation that hCG may be responsible for conferring immunity to tissue rejection not only upon carcinomas but upon the developing embryo itself (Amoroso and Perry, 1975). However, experiments with purified preparations of hCG have produced no consistent support for this hypothesis.

Placental lactogens

These prolactin-like hormones have been definitively identified in primates, ruminants and rodents but are thought to be absent in lagomorphs, carnivores, Equidae and Suidae (Forsyth, 1986, 1991; Ogren and Talamantes, 1988; Talamantes and Ogren, 1988; Byatt *et al.*, 1992). As with the chorionic gonadotrophins, our understanding of the origin and structure of placental lactogens (or chorionic somatomammotrophins) has benefited from the recent application of the techniques of molecular genetics; the human placenta is so rich a source of placental lactogen mRNA sequences that human placental lactogen was the first human gene to be cloned (J. Shine *et al.*, 1977). Following its cloning and sequencing, the structure of the placental lactogen gene was shown to be closely related to that of growth hormone, and five hPL genes and two hGH genes have now been identified in genomic fragments (see Miller and Eberhardt, 1983). Clearly these (and the prolactin) genes have evolved by duplication of a common ancestral precursor gene; sequence analysis and application of rules for gauging mutation rates suggests that prolactin diverged from placental lactogen/growth hormone some 400 million years ago, and that placental lactogen and growth hormone diverged about 65 million years ago. Since

the palaeontological records suggest that amphibia evolved about 400 million years ago, and that mammalian radiation commenced some 85–100 million years ago, these events may have coincided. The chromosomal distribution of these genes also indicates that divergence of prolactin from the ancestral placental lactogen/growth hormone precursor preceded separation of the last two hormones, since the human growth hormone and placental lactogen genes are on chromosome 17, the single prolactin gene being on chromosome 6. The human growth hormone and placental lactogen genes are more than 90% identical in nucleotide sequences (including the introns); the homology between the coding regions of prolactin and growth hormone genes is approximately 40%. Rat and mouse placental lactogens have recently been shown to have two forms, which are secreted consecutively, to give rise to a biphasic secretion pattern during pregnancy (Blank *et al.*, 1977; Soares *et al.*, 1983; Colosi *et al.*, 1987; Kishi *et al.*, 1988; Ogren *et al.*, 1989; Faria *et al.*, 1990; Soares *et al.*, 1991). The molecules have been sequenced and cloned (see Colosi *et al.*, 1989, for references). Their synthesis and secretion by the trophoblast giant cells have been studied *in vivo* and with *in vitro* tissue culture techniques (for references see Soares, 1987; Soares *et al.*, 1991; Yamaguchi *et al.*, 1992).

The other principal group of animals in which placental lactogens have been studied in depth are the ruminants (reviews: Forsyth, 1991; Handwerger, 1991; Byatt *et al.*, 1992); however, less is known of the evolution of these hormones than of hPL, although the ovine, bovine and caprine proteins have been purified (Beckers *et al.*, 1982; Murthy *et al.*, 1982; Arima and Bremel, 1983; Byatt *et al.*, 1986; Currie et al., 1990, McNeilly *et al.*, this volume) and the molecules sequenced and cloned (Colosi *et al.*, 1989). Studies have been made of their secretion (Martal and Djiane, 1977; Taylor *et al.*, 1983; Wallace *et al.*, 1985; Battista *et al.*, 1990) and their interactions with target tissue receptors (Emane *et al.*, 1986; Freemark *et al.*, 1987).

(c) Placental peptide hormones

A number of peptide hormones have been identified in human placental tissue, including LHRH, ACTH, MSH, TSH, oxytocin and vasopressin (see Diczfalusy and Trën, 1961; Brody, 1969; Allen, 1975; Fisher, 1986). Of these, the best characterized is LHRH, which has been identified by immunocytochemistry, radioimmunoassay and bioassay (Siler-Khodr and Khodr, 1978; Tan and Rousseau, 1983). The presence of LHRH in placental tissue has been confirmed by the use made of placental mRNA extracts in the isolation of cDNA clones for the LHRH prohormone (Seeburg and Adelman, 1984). Because of the availability of large quantities of human placental mRNA, and the scarcity of human hypothalamic mRNA, placental mRNA was used to prepare a cDNA library which was screened for LHRH sequences using synthetic oligonucleotides predicted from the LHRH amino acid sequence. It has been shown that the LHRH prohormone in the placenta is processed to produce not only LHRH, as in the hypothalamus, but also GAP (gonadotrophin releasing factor-associated peptide) (Adelman *et al.*, 1986). The role of this product in the placenta is uncertain; although it has been suggested to play a role in the control of hCG secretion, this is not fully established.

(d) Placental secretion of non-hormonal polypeptides

Application of immunological techniques has led during the past two decades to the recognition in man of some 30 pregnancy-associated polypeptides in the circulation during gestation, which have no established endocrine action. Some of these substances are of clinical value in pregnancy diagnosis because of their dependence upon normal

placental or fetal function and some have been used as tumour markers. In addition, a number of proteins have been identified which are not secreted, but which are constituents of the placenta. Although principally identified in man, some have been shown to be present in other species. For reviews see Bohn *et al.* (1983), Klopper and Ahmed (1985), Rosen (1986).

A number of the better characterized placenta-specific proteins are listed in Table 4.4.

Of these, SP_1 (Schwangerschaft protein 1), a glycoprotein (containing 29% carbohydrate) of molecular weight 43 000 daltons and isoelectric point pH 4.1, has been used most as an aid to diagnosis of normal pregnancy and determination of gestational age (see Bennett *et al.*, 1978; Klopper and Ahmed, 1985). This protein is identical with the pregnancy-associated plasma protein (PAPP) C of Lin *et al.* (1974), with pregnancy-specific β_1-glycoprotein (Towler *et al.*, 1976) and with trophoblast-specific β_1-globulin (Tatarinov and Sokolov, 1977). Among the functions suggested for the proteins of Table 4.4 are: SP_1, as an immunosuppressive, steroid binding protein; PAPP-A, a role in the inhibition of the caseinolytic activity of plasmin and in control of the coagulation and complement cascades; and for PP_5, inhibition of the activities of plasmin and trypsin (and therefore a possible role in the control of trophoblast invasiveness).

In addition to these proteins, a number of enzymes circulating in raised concentrations during pregnancy in man have been suggested to be products of secretion or cell lysis by or in the placenta. These include a heat-stable alkaline phosphatase (see Contractor, 1983), oxytocinase and a histaminase.

4.4.2 CELLULAR ORIGINS OF PLACENTAL HORMONES

A variety of methods have been utilized to identify the tissues responsible for secretion of placental polypeptide hormones, including immunocytochemistry and *in situ* hybridization, particularly in human placental preparations. Using these techniques, a number of independent investigations of the synthesis and storage of human placental lactogen (hPL) have localized this hormone to the syncytiotrophoblast (Hoshina *et al.*, 1982; and see Allen, 1975; Patillo *et al.*, 1983, and references therein). Recently, using electron microscope techniques hPL has been localized to membrane-bound syncytiotrophoblast granules (Fujimoto *et al.*, 1986; Morrish *et al.*, 1988; Billingsley and Wooding, 1990) and ruminant PLs exclusively to granules derived from trophectodermal binucleate cells (section 4.3.6b) (Wooding, 1981; C.S. Lee *et al.*, 1986b–d; Wooding and Beckers, 1987; Wooding *et al.*, 1992). In rodents PLs have been demonstrated in trophectodermal giant cells by light microscope immunocytochemistry (Hall and Talamantes, 1984; Campbell *et al.*, 1989; Nieder and Jenne, 1990) and *in situ* hybridization (Duckworth *et al.*, 1990). The electron microscopic localization of hCG to granules (which are morphologically distinct from hPL-containing granules) exclusively in the syncytium has recently established this tissue as the most likely major site of synthesis (Morrish *et al.*, 1987; Billingsley and Wooding, 1990). However, hCG mRNA has been demonstrated in both cytotrophoblast and syncytiotrophoblast by *in situ* hybridization techniques and cells of cytotrophoblast morphology can produce hCG in culture (Boime *et al.*, 1989). This is perhaps not surprising since cytotrophoblast forms syncytiotrophoblast and hCG production is thus merely a precocious expression of a major function.

Methods for the localization of steroids in cell layers have recently been reported using antibodies directed against these small molecules coupled to haptens (G.J. King and Ackerley, 1985). At the light microscope level progesterone and oestrogens were localized exclusively in the syncytiotrophoblast in

Table 4.4 Properties of selected placental polypeptides in man

Protein code	*Molecular weight (kilodaltons)*	*Electrophoretic mobility*	*Earliest time of detection (weeks)*	*Localization in placenta*	*Concentrations*		*Half life post partum*	*Suggested use as indicator of*	
					term placenta (μg/g)	*term peripheral serum (μg/ml)*		*pregnancy failure*	*trophoblast neoplasia*
SP_1	42	$ß_1$	1	ST[b], aminion	50	180	30 hr	Y	Y
PAPP-A	750	$α_2$	12[c]	ST	–	160	51 hr	Y	Y
PAPP-B	1000	$ß_1$	<30[c]	–	–	–	<24 hr	Y	–
PP_5	37	$ß_1$	8	ST	2	0.03	15–30 min	Y	Y
PP_{10}	48	$α_1$	10	ST, amnion	33	3	2–4 days	Y	Y[d]
PP_{11}	44	$α_1$	–[a]	ST	18	–	–	N	Y
PP_{12}	25	$α_1$	5	ST, CT[b], macrophages in amnion and decidua	8	0.17	–	N	–[d]
PP_{13}	30	Albumin	–	–	7	1.0	–	–	–
PP_{17}	30	$ß_1$–$α_2$	–	–	5	1.0	–	–	–

[a]– =not known; [b]ST=syncytiotrophoblast, CT=cytotrophoblast; [c]insensitive detection methods only; [d]non trophoblastic tumour only. Data from Bohn *et al.* Luben (1983); Rosen (1986)

human placenta (Dobashi *et al.*, 1985). However, one would expect that most free steroid will be lost from the tissue during immunocytochemical processing so that a more promising approach is to localize the enzymes unique to steroid synthesis. It should be possible to achieve this with antibodies against them, or by using cDNA probes for their mRNAs. Ultrastructural 'evidence' for steroid synthesis – the presence in a cell of smooth endoplasmic reticulum and lipid droplets – is very unsatisfactory because the correlation is so weak, a reservation which also applies to histochemical methods claiming to localize enzymes such as 3β-hydroxysteroid dehydrogenase. Nonetheless, all the above rather unsatisfactory evidence points to the syncytiotrophoblast as a major site of steroid synthesis in human (Pattillo *et al.*, 1983) and sheep placentas (Ferguson and Christie, 1967) and in trophectodermal giant cells in rodents (Sherman, 1983).

4.4.3 FUNCTIONS OF PLACENTAL HORMONES

The five primary physiological effects of placental hormones listed at the beginning of this section can be provided for by the four main classes of hormone discussed above. However, not all these hormones are secreted by placentas of all species, whereas all five processes are prerequisites for successful intrauterine gestation, so that in some cases they must be provided for by alternative mechanisms.

Broadly speaking, the functions of the placental hormones are as follows, listed here in the order in which they are generally thought to act. In human pregnancy, chorionic gonadotrophin, which is produced immediately following implantation on day 8 or 9 after ovulation (shortly after the 58-cell stage), acts on the corpus luteum to prevent its regression (luteolysis), thereby ensuring a continued supply of progesterone. The requirement for progesterone is met by the placenta after about the sixth or seventh week of gestation (the time of the luteoplacental shift; Csapo, 1977). Progesterone secreted by either the corpus luteum or the conceptus is thought to have several actions necessary for the establishment of pregnancy, including blockade of myometrial prostaglandin production, thereby reducing uterine contractility, stimulation of endometrial gland secretion and inhibition of ovulation, through a negative feedback action on the hypothalamus to prevent FSH secretion.

It should be noted that the luteotrophic role of chorionic gonadotrophin is recognized only in primates; in other species, in particular those with a uterine luteolysin, maintenance of luteal function early in gestation is ensured by inhibition of the secretion of luteolysin by the uterus – an antiluteolytic action. In the sheep, in which this process has been studied in detail, the uterine luteolysin is prostaglandin $F_{2\alpha}$, and its release to the circulation is prevented by secretion by the conceptus of an antiluteolytic protein, termed trophoblastin (Martal *et al.*, 1979; Guillomot *et al.*, 1990) or ovine trophoblast protein 1 (Godkin *et al.*, 1984a; Roberts *et al.*, 1985, 1990; Farin *et al.*, 1989, 1990). The ovine protein, which has recently been shown to be an interferon (Imakawa *et al.*, 1987, 1989; H.J. Stewart *et al.*, 1987), is a relatively low molecular weight compound (16 500 daltons) which is known to interact with endometrial cell membrane receptors (Godkin *et al.*, 1984a,b; H.J. Stewart *et al.*, 1987). A similar protein, bovine trophoblast protein 1, has also been demonstrated in cows (Lifsey *et al.*, 1989).

The principal actions of the other placental steroids, the oestrogens, are thought to be exerted on the myometrium, to cause growth of smooth muscle cells, to accommodate the growing conceptus and to prepare for the onset of parturition (Csapo and Corner, 1952; Michael and Schofield, 1968). In addition, oestrogens have well-defined roles in cellular

proliferation in the endometrium (see Finn and Porter, 1975). Maintenance of high circulating oestrogen concentrations might also be expected to lead to enhanced sensitivity of progesterone-responsive tissues to progesterone, by increasing target tissue progesterone receptor concentrations.

Placental lactogens have been reported to act in a number of ways. Although they are usually considered primarily responsible for mammary development during pregnancy, they most probably act in concert with other hormones to have this effect (Forsyth, 1986), and not all mammals have placental lactogens. Other actions of these hormones include the stimulation of growth factor synthesis (Gluckman *et al.*, 1987; Strain *et al.*, 1987) and effects on maternal metabolism to increase circulating concentrations of metabolites for fetal utilization (Munro, 1980; Chan *et al.*, 1986).

4.5 INTERSPECIFIC EMBRYO TRANSFER AND HYBRID PREGNANCIES: ROLE OF THE PLACENTA

There has recently been increased interest in cross-species hybridization because of the development of embryo transfer techniques and their application to the conservation of endangered species by propagation of rare animals using more readily available surrogate hosts (see G.B. Anderson, 1988). Among notable examples of the application of this procedure are the transfer of the embryos of donkeys (*Equus asinus*), Przewalski horses (*E. przewalskii*) and zebra (*E. burchelli*) to thoroughbred mares (*E. caballus*) (Allen *et al.*, 1986; Summers *et al.*, 1987); *Bos taurus* to *Bos indicus*, *B. gaurus* to *B. taurus* and *Ovis musimon* to *O. aries* (Durrant and Benirschke, 1981); and bongo antelope (*Tragelaptius euryceros*) to African eland (*T. oryk*); (Dresser *et al.*, 1985). Successful application of the technique has been limited to closely related species.

Among the factors limiting interspecific transfers is some form of incompatibility of placentation. In many cases this is likely to include immunological incompatibility. There are two approaches by which this difficulty may be assessed and overcome. Firstly, a consideration of the ability of two species to hybridize may provide an indication of possible success of embryo transfer (Gray, 1971), and there is extensive literature on successful and attempted hybridization experiments. A second approach is in the transfer of inner cell mass cells (of the rare species) within the trophectoderm cells of the host; this technique, which was pioneered as means of identification and manipulation of cell lines in the developing embryo (Gardner, 1975), has been exploited most successfully in crosses of *M. musculus* x *M. caroli* (Rossant and Frels, 1980; Croy *et al.*, 1982; Crepeau *et al.*, 1989).

Because of the large amount of work done with them, sheep x goat crosses are of particular interest in relation to interspecies hybridization. While most authors agree that these hybrid embryos die between the fourth and fifth week of gestation, there have been reports of the birth of live offspring: Bunch *et al.* (1976) described the fertile twin offspring of a female 'Spanish' goat (2n = 60) and a Barbados sheep ram (2n = 54). The hybrids had an intermediate phenotype and chromosome number (2n = 57), and the female progeny was bred back to a Barbados ram, with the resulting twins having 2n = 55. It has been suggested on the basis of morphological examination of the site of placental attachment (and on other grounds) that pregnancy failure in cases of unsuccessful hybridization is caused by immunological rejection (McGovern, 1973; Benirschke, 1983); this is consistent with observations of Croy *et al.* (1982) and Crepeau *et al.* (1989) on rejection of *M. caroli* embryos in *M. musculus*, and with the fact that prior immunization of goats against sheep tissue reduces the length of fetal survival in crosses of these species (McGovern, 1973). Endocrine factors are unlikely to account for interspecies incom-

patibility in sheep x goat hybrids; simultaneous homospecific embryo transfer does not improve the outcome of sheep × goat hybridization, so failure to maintain the corpus luteum is unlikely to be involved. Furthermore, crosses between *O. aries* (2n = 54) and *Ammotragus lervia* (Barbary sheep, 2n = 58) are generally unsuccessful, although the latter species, unlike the goat but like the sheep, has a progesterone-secreting placenta (Flint *et al.*, 1983). Successful goat × sheep chimaeras have been produced by heterologous inner cell mass transfer with homologous trophectoderm (Fehilly *et al.*, 1984; Meinecke-Tillmann, 1984), but there is no information on placental endocrinology in these pregnancies. Transfer of Dall's sheep embryos to the uteri of domestic ewes has generally been unsuccessful (Buckrell *et al.*, 1990). Clearly an exciting prospect in this field is the possibility of storing gametes or even somatic DNA from endangered species; indeed it may one day be possible to revive extinct species using DNA recovered from their carcasses – DNA sequences from extinct animals have already been published.

4.6 VIVIPARITY

4.6.1 FUNCTIONAL REQUIREMENTS

The adoption of viviparity has involved the acquisition by the embryonic membranes of a number of functions not associated with them in the oviparous condition. However, there are functions, such as gaseous exchange, that are evidently necessary in oviparity and viviparity, quite regardless of the length of time for which the embryo is retained in the uterus. The placental functions associated with viviparity, which include endocrine functions (Amoroso *et al.*, 1979) and those involved in the absorption of uterine secretions not found in the egg, should therefore be seen as being superimposed upon those that are more primitive. Unfortunately, too little is known about the intrauterine environment and maternal physiology, in too few species, to permit a synthesis of laws accounting for the various morphological and functional characteristics of fetal membranes in vertebrates in general. Species differences further complicate the issue. Nonetheless, some generalizations can be made.

The need to provide large amounts of yolk *in utero*, for the development of the embryo as occurred during the evolution of elasmobranchs, required the yolk sac enveloping the yolk to become well vascularized, larger and longer lasting. This membrane, being derived from the fetal gut, is well adapted to convey extraembryonic material in the yolk into the fetal circulation by pinocytosis. It serves as an intrauterine organ of exchange in viviparous species in this order. Following colonization of the land, it was necessary for these early vertebrates either to become viviparous or to develop eggs capable of surviving out of water. The adaptations resulting from these pressures are the amnion and the allantois, the former presumably coming into being as a means of providing the embryo with its own private sea, the latter initially as a repository for excretory products and an organ of gaseous exchange. The development of these membranes provided a means for carrying out functions not undertaken by the yolk sac, perhaps because it was too highly specialized for absorption of nutrient of a particular type. The yolk sac is capable of carrying out sufficient gaseous exchange to support early embryonic development in the avian egg; but later in development, when the fetal needs increase, the allantois takes over this role. The allantois is also involved in uptake of nutrients; in the chick, the allantoic capillaries deeply indent the chorionic ectoderm, with which the embryo is surrounded. This not only increases the capacity for gaseous exchange, but also provides a means for the absorption, by the chorioallantois, of large quantities of calcium from the egg shell. Thus in the highly developed avian egg there

is a vascularized yolk sac membrane capable of sustaining gaseous exchange early in development, but mainly adapted for pinocytotic absorption of yolk and a chorioallantoic membrane subsequently developed for gaseous exchange and absorption of mineral.

4.6.2 THE EVOLUTION OF VIVIPARITY

Placentation is associated with viviparity. It is relevant therefore to a discussion of placentation to enquire as to the nature of viviparity. It should be noted from the outset that the terminology – oviparity, ovoviviparity, viviparity – is restrictive, in that each of these categories of parity includes a wide range of forms; for instance the term oviparity covers oviposition of both fertilized and unfertilized eggs, and viviparous organisms include those with both external and internal fertilization (see Guillette, 1991). Furthermore, there are other, equally revealing, characteristics that need to be considered when seeking to categorize forms of reproduction (for example see Crews, 1992). Forms of viviparity and ovoviviparity have been considered in more detail by Mossman (1987) and Clutton-Brock (1991).

The European olm (*Proteus anguinis*), a blind, colourless salamander of the Italian Alps, can be either oviparous or viviparous. When the ambient temperature rises above about 12°C, all of the eggs ovulated by this amphibian (approximately 60) are laid, though not all develop; at lower temperatures a single egg develops in each oviduct, and live young are born. This change in reproductive pattern occurs under artificial conditions, as well as in nature, and has been studied in the laboratory. There are also reptiles which are believed to be both viviparous and oviparous but in different geographic locations: examples are the Mexican lizard, *Sceloporus aeneus* (viviparous in some sites in southern Mexico, oviparous in others); the skink, *Mabuya quinquetaeniata* (viviparous in South America, oviparous elsewhere); the iguana, *Sceloporus variabilis* (viviparous at high altitudes in Veracruz, Mexico); and the vipers *Echis carinata* and *Aspis vipera* (oviparous in some locations, viviparous in others; see Hogarth, 1976; Guillette, 1981).

The existence of animals which can be both oviparous and vivparous or in which closely related species have both characteristics emphasizes one of the most striking characteristics of viviparity: that it is a readily adopted condition. This is further underlined by the wide range of viviparous invertebrates and vertebrates, the large number of times the condition has evolved in different phyla and the variety of methods used (see Shine and Guillette, 1988). That viviparous species evolved from oviparous species is shown by the existence of egg-teeth in embryos of viviparous reptiles.

Viviparity occurs in all classes of amniote vertebrates except birds and takes a number of forms; the organ most frequently utilized for embryonic development is the oviduct or uterus, but there are examples of embryonic development occurring in the ovary (notably in the teleost fishes, in which gestation may occur either in the ovarian follicles as in the family Poeciliidae or in a chamber within the ovary, following ovulation, as in the blenny, *Zoarces viviparus*). Gestation may also occur in the mouth (as in the catfish, *Galeichthys*); in the stomach (as in the Australian anuran amphibian, *Rheobatrachus silus*); and in skin pouches (as in the Surinam toad, *Pipa surinamensis*). In these cases the embryos develop specialized organs for nutrient exchange, including vascular tails and outgrowths from the gills and pericardium; the embryonic membranes of the amniote egg are not utilized for this purpose. These forms of gestation are dealt with in detail in sections 4.31, 4.32 and 4.34 above.

It is difficult to generalize about the forms of viviparity adopted by invertebrates because, as with lower vertebrates, a wide range of organs is utilized. But there are

examples of structures specialized for nutrient exchange in invertebrates, which fulfil the criteria for placentas, as for example in the marine tunicate, *Salpa* (Bone *et al.*, 1985).

The principal advantages conferred by viviparous reproduction appear to lie in the protection the condition provides for the developing embryo against fluctuations in environmental conditions. This is particularly important for terrestrial species, which must if oviparous either return to water to breed or lay an egg protected against desiccation. The main environmental advantages are usually seen as those relating to temperature, availability of water, provision of food for emerging young, selection of a site for birth and protection against predation. The complicated behaviour patterns evolved by birds to ensure incubation of the eggs demonstrate the lengths to which oviparous species must go to provide some degree of homeostasis for their developing offspring. It is not necessarily true that the birth of live young leads to a greater degree of post-partum maternal care: the existence of the monotremes, oviparous mammals, emphasizes the fact that lactation and viviparity are not inseparable conditions. Lactation and endothermy evolved before viviparity in mammals. Nonetheless, it does appear that viviparity may be utilized by species colonizing environmentally extreme locations, for example at high latitudes and altitudes.

Although internal fertilization does not invariably lead to viviparity, one prerequisite for viviparity in amniote vertebrates is a means of transferring spermatozoa with concomitant internal fertilization, and structures for providing and absorbing maternal nutrients at the site at which development occurs. Internal fertilization confers advantages of its own, in as much as it reduces the risks of loss of spermatozoa and interspecies fertilization. It does not, however, invariably involve an intromittent organ; in the salamanders and newts the spermatozoa are frequently deposited in a protective capsule (spermatophore) which the female picks up with the lips of the cloaca.

While organs evolved for internal fertilization show little variability, embryonic absorptive organs, in contrast, take many forms, particularly in amphibia, in which they include specially adapted gills (as in *Gastrotheca*, in which development occurs in maternal skin pouches), and vascularized tails (as in the tadpole of the toad *Pipa surinamensis*, also incubated in a skin pouch). The chorioallantoic placenta provides an amniote example of organs specialized for this role.

With the development of maternal structures specialized for embryonic nutrition the nature of the material secreted (uterine milk) becomes more complex, the metabolic cost to the mother becomes more severe and the weight gain by the conceptus more dramatic (Table 4.2). Furthermore, the dependence of the uterus on endocrine stimulation by ovarian or embryonic hormones also becomes more important, and in both oviparous elasmobranchs and viviparous species with a simple serous type of uterine milk the uterus responds to administered oestrogen with increased vascularization and growth of the oviduct or uterus.

One of the reasons for the relative ease with which viviparity is adopted, particularly among amniote vertebrates, is the nature of the embryonic membranes of the egg, which are the evolutionary precursors of the embryonic membranes of the mammalian placenta (Figure 4.1). These membranes, which are adapted in the egg for gaseous exchange and the absorption of nutrients in the form of yolk, retain these functions in viviparity and take on others. In the mother, in addition, similar structures exist in oviparous and viviparous species for the provision of nutrients to the developing embryo, in the form of either yolk (in oviparity) or histotroph (in viviparity). Thus, limited anatomical alterations are required for the adoption of viviparity, once internal fertilization is achieved. In general, the degree to which the embry-

onic membranes have evolved is related to the dependence of the developing embryo on nutrients derived from the mother. Where there is no nutrient exchange, as in ovoviviparous species, there is no development of egg membranes and, in fact, no loss of the shell membrane. The evolution of placentation in vertebrates and the concomitant decline in the quantity of yolk in the egg parallels the ability to absorb maternal nutrients. The eggs of all viviparous reptiles are yolk rich. Development of placental endocrine function provides a means by which the conceptus is able to affect maternal metabolism in order to ensure mobilization of a sufficient supply of nutrient (for instance through secretion of a placental lactogen).

What then are the most important adaptations associated with viviparous reproduction? A potentially important change involves the corpus luteum and its secretions; as Amoroso (1981) commented: 'although the exact steps through which the corpus luteum was incorporated as an endocrine gland may remain obscure, there can be little doubt that this adaptation was the most important contributing factor in the evolution of viviparity in mammals'. It seems most probable that the existence of progesterone, secreted by a corpus luteum in oviparous species, was exploited in the evolution of viviparity for the purposes of continuing the provision of embryonic nutrition: an example of Medawar's proposition (Medawar, 1953) that 'it is not hormones which have evolved but the uses to which they are put'. Comparison of circulating progesterone levels during the reproductive season in the oviparous turtle, *Chrysemys picta* with that in the viviparous snake, *Natrix fasciata*, emphasizes the importance of post-ovulatory progesterone in the viviparous condition (Porter *et al.*, 1982).

Guillette and his colleagues (Guillette, 1982, 1991; Shine and Guillette, 1988) have suggested that the evolution of viviparity involves selection pressures acting on phenotypic variability in egg retention, with animals in extreme environments retaining oviductal eggs for progressively longer periods. A concomitant thinning of the shell, to allow gaseous exhange, accompanied by increasing maternal nutrient secretion, eventually leads from oviparity, via intermediate ovoviviparous stages, to viviparity. They propose that progesterone, possibly secreted by the adrenal glands, as well as the corpora lutea, causes prolonged retention in relatively stressful conditions, and the effects of progesterone on the mammalian uterus (increased nutrient secretion, decreased mobility and reduced prostaglandin secretion) are consistent with this hypothesis.

Among squamates (lizards and snakes), in which this hypothesis has been most fully developed, oviparous species retain their eggs *in utero* for up to half the period of embryonic development, and post-ovulatory corpora lutea occur in all vertebrate groups (though they are transitory in cyclostomes and birds), and are steroidogenic in all species of reptile. Reptilian corpora lutea are susceptible to regression by prostaglandin $F_{2\alpha}$ (Guillette *et al.*, 1984), and prostaglandin $F_{2\alpha}$ is synthesized by the oviduct in at least one viviparous lizard (Guillette *et al.*, 1988).

All three effects of progesterone facilitate prolonged egg retention and continued luteal function (by delaying luteolysis), and this may explain the apparent ease with which prolonged egg retention has resulted in viviparity in many different species.

However, not all aspects of this hypothesis are supported by experimental data, and although attractive in many taxa (especially the lizards), there is contradictory evidence. Experimental evidence is not available to directly link prolonged uterine egg retention with high levels of endogenous progesterone, or to confirm that raised progesterone secretion can be a direct response to adverse environmental conditions (see Shine and Guillette, 1988), although these questions are amenable to investigation. The possible contribution of relaxin, also secreted by the cor-

pora lutea, and a potent inhibitor of uterine contractility in several species of mammal, should also be considered in this context (see Porter *et al.*, 1982).

The adoption of viviparity, and the establishment of close contact between the developing embryo and the mother, provides an opportunity for the conceptus to influence maternal physiology through endocrine mechanisms. This occurs early in pregnancy in many species, when the conceptus secretes substances which ensure the prolongation of corpus luteum function beyond the period of the oestrous cycle; the time at which this occurs is known as the time of the maternal recognition of pregnancy and it is a well-characterized process, particularly in those species (e.g. artiodactyls) in which it occurs before the attachment of the blastocyst to the uterine endometrium. A number of substances of embryonic origin are responsible for this process, including an interferon (the antiluteolysin which controls uterine production of prostaglandin $F_{2\alpha}$ in ruminants), chorionic gonadotrophins (in primates) and perhaps oestrogens (in pigs). Another substance capable of controlling corpus luteum function is prolactin, which is produced in the decidua, and has been suggested to affect fluid transport across the embryonic membranes (see Porter *et al.*, 1982). Clearly the coexistence in the monotremes of oviparity and lactation rules out the possibility that mammary development and lactation depend exclusively on hormones secreted by the placenta; in fact in some eutherian mammals (e.g. pigs) no mammogenic hormone has been identified in the placenta, although many species, particularly rodents, artiodactyls and primates, produce placental lactogens.

The parallels between oviposition and parturition, particularly as regards similarities in their endocrine control, have been drawn previously (e.g. Toth *et al.*, 1983), and it is tempting to view parturition as oviposition delayed, not least because the same hormones are involved in both processes (see R.E. Jones and Guillette, 1982). The frequent occurrence of a post-partum (as in rodents) or prepartum (as in hares) oestrus and ovulation further emphasizes the similarity: progesterone, oestrogens, prostaglandins and the neurohypophysial peptides, oxytocin or vasotocin, clearly are involved in both cases. However, the trigger to parturition, which in many species arises in the fetus (e.g. fetal glucocorticoid; see section 4.4.1a) differs from that causing oviposition, which resides in the hypothalamus; in fact it may be more appropriate from a fetal point of view to consider parturition as akin to hatching.

In summary, the prerequisites for vertebrate viviparity presumably include the following: (a) acquisition of internal fertilization; (b) development of a corpus luteum which controls endometrial glandular secretion of egg nutrients; (c) either luteal production of a compound such as relaxin, which blocks oviductal smooth muscle, or the acquisition, by oviductal smooth muscle, of the block to contractility induced by progesterone; (d) thinning of the tertiary layers surrounding the egg, and loss of the egg shell, accompanied by a reduction in the number of uterine glands; (e) embryonic absorption of maternal nutrients; (f) a block to immunological rejection of the conceptus; (g) a mechanism for ensuring parturition. The principal disadvantage with this form of reproduction is that the size of the fetal brain at birth, which may have consequences for the intelligence of the offspring, is limited by the size of the mother's pelvic girdle, and it may be no coincidence that relaxin, which reduces uterine contractility, and thereby prolongs uterine retention of the conceptus, also leads to separation of the pelvic symphyses. This is particularly marked in species such as the guinea pig, with large and precocious young.

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REFERENCES

Adelman, J.P., Mason, A.J., Hayflick, J.S. and Seeburg, P.H. (1986) Isolation of the gene and hypothalamic cDNA for the common precursor of gonadotropin-releasing hormone and prolactin release-inhibiting factor in human and rat. *Proceedings of the National Academy of Sciences of the USA*, **83**, 179–183.

Aitken, R.J. (1974) Delayed implantation in roe deer. *Journal of Reproduction and Fertility*, **39**, 225–233.

Al-Timimi, A. and Fox, H. (1986) Immunohistochemical localisation of FSH, LH, GH, ACTH and PRL in the human placenta. *Placenta*, **7**, 163–172.

Alexander, G. (1964) Studies on the placenta of the sheep (*Ovis aries* L.): effect of surgical reduction in the number of caruncles. *Journal of Reproduction and Fertility*, **7**, 307–322.

Alexander, G. and Williams, D. (1966) Progesterone and placental development in the sheep. *Journal of Endocrinology*, **34**, 241–245.

Alexander, G. and Williams, D. (1968) Hormonal control of amniotic and allantoic fluid volume in ovariectomized sheep. *Journal of Endocrinology*, **41**, 477–485.

Allen, W.R. (1975) Endocrine functions of the placenta, in *Comparative Placentation* (ed. D. Steven), Academic Press, London, pp. 214–267.

Allen, W.R. (1982) Immunological aspects of the endometrial cup reaction and the effect of xenogeneic pregnancy in horses and donkeys. *Journal of Reproduction and Fertility*, **31**, 57–94.

Allen, W.R. and Moor, R.M. (1972) The origin of the equine endometrial cups. I. Production of PMSG by fetal trophoblast cells. *Journal of Reproduction and Fertility*, **29**, 313–316.

Allen, W.R., Hamilton, D.W. and Moor, R.M. (1973) Origin of equine endometrial cups. II. Invasion of the endometrium by trophoblast. *Anatomical Record*, **177**, 485–502.

Allen, W.R., Kydd, J.H. and Antczak, D.F. (1986) Successful applications of immunotherapeutics to a model of pregnancy failure in equids, in *Reproductive Immunology* (eds D.A. Clark and B.A. Croy), Elsevier Science, Amsterdam, 253–260.

Allen, W.R., Kydd, J.H., Boyle, M.S. and Antczak, D.F. (1987) Extraspecific donkey x horse pregnancy as a model for early fetal death. *Journal of Reproduction and Fertility*, Suppl. 35, 197–209.

Amoroso, E.C. (1952) Placentation, in *Marshall's Physiology of Reproduction*, Vol. II Chapter 15, Longman, Green and Co., London, pp. 127–311.

Amoroso, E.C. (1960) Viviparity in fishes. *Symposia of the Zoological Society of London*, **1**, 153–181.

Amoroso, E.C. (1981) Viviparity, in *Cellular and Molecular Aspects of Implantation* (eds S.R. Glasser and D.H. Bullock), Plenum Press, New York, pp. 3–25.

Amoroso, E.C. and Perry, J.S. (1975) The existence during gestation of an immunological buffer zone at the interface between maternal and foetal tissues. *Philosophical Transactions of the Royal Society*, **B 271**, 343–361.

Amoroso, E.C., Heap, R.B. and Renfree, M.B. (1979) Hormones and the evolution of viviparity (ed. E.J.W. Barrington), *Hormones and Evolution*, Academic Press, New York.

Anderson, A.B.M., Flint, A.P.F. and Turnbull, A.C. (1975) Mechanism of action of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. *Journal of Endocrinology*, **66**, 61–70.

Anderson, D. and Faber, J.J. (1982) Regulation of fetal placental blood flow in the lamb. *American Journal of Physiology*, **247**, R567–R574.

Anderson, G.B. (1988) Interspecific pregnancy: barriers and prospects. *Biology of Reproduction*, **38**, 1–15.

Anderson, J.W. (1969) Ultrastructure of the placenta and fetal membranes of the dog. I. The placental labyrinth. *Anatomical Record*, **165**, 15–36.

Anderson, J.W. and Wimsatt, W.A. (1963) Placentation and fetal membranes of the Central

American Noctilionid bat Noctilio labialis minor. *American Journal of Anatomy*, **112**, 181–202

Anderson, T.L. and Hoffman, L.H. (1984) Alterations in epithelial glycocalyx of rabbit uteri during early pseudopregnancy and pregnancy and following ovariectomy. *American Journal of Anatomy*, **171**, 321–334.

Anderson, T.L., Olson, G.E. and Hoffman, L.H. (1986) Stage specific alterations in the apical membrane glycoproteins of endometrial epithelial cells related to implantation in rabbits. *Biology of Reproduction*, **34**, 701–720.

Aoki, A., Metz, J. and Forssman, W.G. (1978) Studies on the ultrastructural and permeability of the haemotrichorial placenta. II. Fetal capillaries and tracer administration into the fetal blood circulation. *Cell and Tissue Research*, **192**, 409–422.

Aplin, J.D. (1991) Review: Implantation, trophoblast differentiation and hemochorial placentation, mechanistic evidence *in vivo* and *in vitro*. *Journal of Cell Science*, **99**, 681–692.

Arima, Y. and Bremel, R.D. (1983) Purification and characterisation of bovine placental lactogen. *Endocrinology*, **113**, 2186–2194.

Aschheim, S. and Zondek, B. (1927) Hypophysen vorderlappenhormon und ovarial–hormon im Harn von schwangeren. *Klinische Wochenschrift*, **6**, 1322.

Assheton, R. (1906) The morphology of the ungulate placenta, particularly the development of that organ in the sheep and notes upon the placenta of the elephant and hyrax. *Philosophical Transactions of the Royal Society*, **B 198**, 143–315.

Bacon, B.J., Gilbert, R.D., Kaufmann, P., Smith, A.D., Trevino, F.T. and Longo, L.D. (1984) Placental anatomy and diffusing capacity in guinea pigs following long–term maternal hypoxia. *Placenta*, **5**, 475–488.

Bainter, K. (1986) Intestinal absorption of macromolecules and immune transmission from mother to young. CRC Press, Florida.

Bambra, C.S., Lynch, S.S., Foxcroft, G.R., Robinson, G. and Amoroso, E.C. (1984) Purification and characterization of guinea-pig chorionic gonadotrophin. *Journal of Reproduction and Fertility*, **71**, 227–233.

Barcroft, J. (1933) *Researches on Pre-natal Life*. Blackwell Scientific Publications, Oxford.

Barcroft, J. and Barron, D.H. (1946) Observations on the form and relations of the maternal and fetal vessels in the placenta of the sheep. *Anatomical Record*, **94**, 569–595.

Barlow, P.W. and Sherman, M.I. (1972) The biochemistry of differentiation of mouse trophoblast: studies on polyploidy. *Journal of Experimental Embryology and Medicine*, **27**, 447–465.

Barnes, R.J., Comline, R.S., Silver, M. and Steven, D.H. (1976) Ultrastructural changes in the placenta of the ewe after fetal hypophysectomy or adrenalectomy. *Journal of Physiology*, **263**, 173–174.

Barrau, M.D., Abel, J.H., Torbit, C.A. and Tietz, W.J. (1975) Development of the implantation chamber in the pregnant bitch. *American Journal of Anatomy*, **143**, 115–130.

Bass, F., Krane, E.J., Mallon, K., Nathanielz, P.W. and Steven, D.H. (1977) Mobilisation of binucleate cells in the placenta of the ewe after foetal pituitary stalk section. *Journal of Endocrinology*, **73**, 36–37.

Bassett, J.M. (1991) Current perspectives on placental development and its integration with fetal growth. *Proceedings of the Nutrition Society*, **50**, 311–319.

Bassett, J.M., Oxborrow, T.J., Smith, I.D. and Thorburn, G.D. (1969) The concentration of progesterone in the peripheral plasma of the pregnant ewe. *Journal of Endocrinology*, **45**, 449–457.

Battaglia, F.C. (1992) New concepts in fetal and placental amino acid metabolism. *Journal of Animal Science*, **70**, 3258–3270.

Battaglia, F.C. and Meschia, G. (1973) Foetal metabolism and substrate utilization, in *Proceedings of the Sir Joseph Barcroft Centenary Symposium* (eds K.S. Comline and K.W. Cross), Cambridge University Press, pp. 382–397.

Battaglia, F.C. and Meschia, G. (1978) Principal substrates of fetal metabolism. *Physiological Reviews*, **58**, 499–527.

Battaglia, F.C. and Meschia, G. (1986) *An Introduction to Fetal Physiology*, Academic Press, Orlando, Florida.

Battista, P.J., Bell, A.W., Deaver, D.R. and Currie, W.B. (1990) Differential control of placental lactogen release and progesterone production by ovine placental tissue *in vitro*. *Placenta*, **11**, 337–348.

Bauchot, R. (1965) La placentation chez les reptiles. *Annales Biologia*, **4**, 547–575.

Baur, R. (1972) Quantitative analyse des wachstums der Zottenoberfläche bei der Placenta des Rindes und des Menschen. *Zeitschrift fur Anatomie Entwicklungsgeschichte*, **136**, 87–97.

Baur, R. (1977) Morphometry of the placental

exchange area. *Advances in Anatomy Embryology and Cell Biology*, **53**, 1–63.

Bazer, F.W. and First, N.L. (1983) Pregnancy and parturition. *Journal of Animal Science*, **57**, Suppl. 2, 425–460.

Bazer, F.W., Marengo, S.R., Geisert, R.D. and Thatcher, W.W. (1984) Exocrine versus endocrine secretion of $PGF_{2\alpha}$ in the control of pregnancy in swine. *Animal Reproduction Science*, **7**, 115–132.

Bazer, F.W., Vallet, J.L., Roberts, R.M., Sharp, D.C. and Thatcher, W.W. (1986) Role of conceptus secretory products in establishment of pregnancy. *Journal of Reproduction and Fertility*, **76**, 841–850.

Bazer, F.W., Vallet, J.L., Harvey, J.P., Gross, T.S. and Thatcher, W.W. (1989) Comparative aspects of maternal recognition of pregnancy between sheep and pigs. *Journal of Reproduction and Fertility*, Suppl. 37, 85–89.

Beaulaton, J. and Lockshin, R.A. (1982) The relation of programmed cell death to development and reproduction: Comparative studies and an attempt at classification. *International Review of Cytology*, **79**, 215–235.

Becher, H. (1931) Placenta und uterus schleimhaut von Tamandua tetradactyla (Myrmecophaga). *Morphologisches Jahrbucher*, **67**, 381–458.

Beck, F. and Lowe, J.R. (1972) The stimulation of a maternal pregnancy reaction in the ferret. *Journal of Anatomy*, **111**, 333.

Beckers, J.F., DeCoster, R., Wouter-Ballman, P., Fromant Lienard, Ch., Vander Zwalmen, P. and Ecters, F. (1982) Dosage radioimmunologique de l'hormone placentaire somatotrope et mammotrope bovine. *Annales Médecine Vétérinaire*, **126**, 9–21.

Bell, A.W. (1984) Factors controlling placental and fetal growth and their effects on future production, in *Reproduction in Sheep* (eds D.H. Lindsay and D.T. Pearce) Cambridge University Press, pp. 144–152.

Bell, A.W. (1991) The whole animal – pregnancy and fetal metabolism, in *Quantitative Aspects of Ruminant Digestion and Metabolism* (eds J.M. Forbes and J. France), Butterworths, London, pp. 100–145.

Bell, C. (1972) Autonomic nervous control of reproduction: circulatory and other factors. *Pharmacological Reviews*, **24**, 657–736.

Bell, C. (1974) Control of uterine blood flow in pregnancy. *Medical Biology*, **52**, 219–228.

Bell, S.C. (1979) Immunochemical identity of decidualisation associated protein and $\alpha_2$acute phase macroglobulin in the pregnant rat. *Journal of Reproductive Immunology*, **1**, 193–206.

Bell, S.C. (1983) Decidualisation: regional differentiation and associated functions. *Oxford Review of Reproductive Biology*, **5**, 220–271.

Bell, S.C., Patel, S.R., Jackson, J.A. and Waites, G.T. (1988) Major secretory protein of human decidualised endometrium is an IGF binding protein. *Journal of Endocrinology*, **118**, 317–328.

Ben David, E. and Shemesh, M. (1990) Localisation of cytoplasmic side chain cleavage enzyme in the bovine placentome using the protein A gold technique. *Biology of Reproduction*, **42**, 131–138.

Ben Rafael, Z. and Orvieto, R. (1992) Cytokines: involvement in reproduction. *Fertility and Sterility*, **58**, 1093–1099.

Benirschke, K. (1983) Placentation. *Journal of Experimental Biology*, **200**, 385–389.

Bennett, M.J., Grudzinskas, J.G., Gordon, Y.B. and Turnbull, A.C. (1978) Circulating levels of alpha-fetoprotein and pregnancy specific β, glycoprotein in pregnancies without an embryo. *British Journal of Obstetrics and Gynaecology*, **85**, 348–350.

Betteridge, K.J. (1989) The structure and function of the equine capsule in relation to embryo manipulation and transfer. *Equine Veterinary Journal*, Suppl. 8, 91–100.

Bevilacqua, E.M.A.F. and Abrahamsohn, P.A. (1989) Trophoblast invasion during implantation of the mouse embryo. *Archivos de Biologia y Medicina Experimentales*, **22**, 107–118.

Bhavnani, B.R. and Woolever, C.A. (1978) Formation of steroids by the pregnant mare. VI. Metabolism of [^{3}H]dehydroepiandrosterone injected into the fetus. *Endocrinology*, **103**, 2291–2298.

Bhiwgade, D.A. (1990) Comparative electron microscopy of the chorioallantoic placental barrier in some Indian Chiroptera. *Acta Anatomica*, **138**, 302–317.

Billingsley, S.A. and Wooding, F.B.P. (1990) An immunogold cryoultrastructural study of sites of synthesis and storage of chorionic gonadotrophin and placental lactogen in human syncytiotrophoblast. *Cell and Tissue Research*, **261**, 375–382.

Billington, W.D. and Bell, S.C. (1983) Immunology of mouse trophoblast, in *Biology of Trophoblast* (eds C. Loke and A. Whyte), Elsevier, Amsterdam, pp. 571–594.

Bissonnette, J.M. (1981) Studies *in vivo* of glucose transfer across the guinea-pig placenta. *Placenta*, Suppl. 2, 155–162.

Bissonnette, J.M. and Farrell, R.C. (1973) Pressure–flow and pressure–volume relationships in the fetal placental circulation. *Journal of Applied Physiology*, **35**, 355–360.

Bissonnette, J.M. and Wickham, W.K. (1977) Placental diffusing capacity for carbon monoxide in unanesthetized guinea pigs. *Respiration Physiology*, **31**, 161–168.

Bjorkman, N.H. (1968) Fine structure of cryptal and trophoblastic giant cells in the bovine placentome. *Journal of Ultrastructural Research*, **24**, 249–258.

Bjorkman, N.H. (1970) *An Atlas of Placental Fine Structure*, Williams & Wilkins, Baltimore, USA.

Bjorkman, N.H. and Bloom, G. (1957) On the fine structure of the foetal–maternal junction in the bovine placentome. *Zeitschrift Zellforschung*, **45**, 649–659.

Bjorkman, N.H. and Wimsatt, W.A. (1968) The allantoic placenta of the vampire bat (Desmodus rotundus marinus): A reinterpretation of its structure based on electron microscopic observations. *Anatomical Record*, **162**, 83–98.

Blackburn, D.G. (1993) Chorioallantoic placentation in Squamate reptiles: structure, function, development and evolution. *Journal of Experimental Zoology*, **266**, 414–430.

Blackburn, D.G., Vitt, L.J. and Beuchat, C.A. (1984) Eutherian like reproductive specialisation in a viviparous reptile. *Proceedings of the National Academy of Sciences of the USA*, **81**, 4860–4863.

Blackburn, D.G., Osteen, K.G., Winfrey, V.P. and Hoffman, L.H. (1989) Obplacental giant cells of the domestic rabbit. Development, morphology and intermediate filament composition. *Journal of Morphology*, **202**, 185–203.

Blank, M.S., Chan, J.S.D. and Friesen, H.G. (1977) Placental lactogens, new developments. *Journal of Steroid Biochemistry*, **8**, 403–414.

Blankenship, T.N. and Given, R.L. (1992) Penetration of the uterine epithelial basement membrane during blastocyst implantation in the mouse. *Anatomical Record*, **233**, 196–204.

Blankenship, T.N., Given, R.L. and Parkening, T.A. (1990) Blastocyst implantation in the chinese hamster (*Cricetulus griseus*). *American Journal of Anatomy*, **187**, 137–157.

Blankenship, T.N., Enders, A.C. and King, B.F. (1992) Distribution of laminin, type IV collagen and fibronectin in the cell columns and trophoblast shell of early macaque placentas. *Cell and Tissue Research*, **270**, 241–248.

Bleier, W.J. (1975) Early embryology and implantation in the California leafnosed bat Macrotus californicus. *Anatomical Record*, **182**, 237–254.

Bodley, H.D. (1974) Ultrastructural development of the chorioallantoic placental barrier in the bat Macrotus waterhousii. *Anatomical Record*, **180**, 351–368.

Bohn, H., Dati, F. and Lüben, G. (1983) Human trophoblast specific products other than hormones, in *Biology of Trophoblast* (eds Y.W. Loke and A. Whyte), Elsevier Science Publishers, Amsterdam, pp.317–352.

Boime, I., Otani, T. and Chaplin, D. (1989) Regulation of the hCG β subunit genes. *Journal of Reproduction and Fertility*, Suppl. 37, 1–10.

Bone, Q., Pulsford, A.L. and Amoroso, E.C. (1985) The placenta of the salp (*Tunicata: Thaliacea*). *Placenta*, **6**, 53–64.

Boshier, D.P. (1969) A histological and histochemical examination of implantation and early placentome formation in sheep. *Journal of Reproduction and Fertility*, **19**, 51–61.

Boshier, D.P. (1970) The pontamine blue reaction in pregnant sheep uteri. *Journal of Reproduction and Fertility*, **22**, 595–596.

Boshier, D.P. and Holloway, H. (1977) The sheep trophoblast and placental function: an ultrastructural study. *Journal of Anatomy*, **124**, 287–298.

Boving, B. (1971) Biomechanism of implantation, in *Biology of the Blastocyst* (ed. R.J. Blandau), University of Chicago Press.

Bower, D.J. (1987) Chromosome organisation in polyploid mouse trophoblast nuclei. *Chromosoma*, **95**, 76–80.

Boyd, J.D. and Hamilton, W.J. (1970) *The Human Placenta*, Heffers, Cambridge.

Boyd, R.D., Morriss Jr., F.H., Meschia, G., Makowski, E.L. and Battaglia, F.C. (1973) Growth of glucose and oxygen uptakes by fetuses of fed and starved ewes. *American Journal of Physiology*, **225**, 897–902.

Bradbury, S., Billington, W.D. and Kirby, D.R.S. (1965) A histochemical and electron microscopical study of the fibrinoid of the mouse placenta. *Journal of the Royal Microscopical Society*, **84**, 199–211.

Brambel, C.E. (1933) Allantochorionic differentiations of the pig studied morphologically and histochemically. *American Journal of Anatomy*, **51**, 397–459.

Brambell, F.W.R. (1966) The transmission of passive immunity from mother to young and the catabolism of immunoglobulins. *Lancet*, **ii**, 1087–1093.

Brambell, F.W.R. (1970) The transmission of passive immunity from mother to young. *Frontiers of Biology*, Vol. 18, North Holland Press, Amsterdam.

Braverman, M.B., Bagni, A., DeZiegler, D., Den, T. and Gurpide, E. (1984) Isolation of PRL producing cells from first and second trimester decidua. *Journal of Clinical Endocrinology and Medicine*, **58**, 521–528.

Brierley, J. and Clark, D.A. (1987) Characterisation of hormone dependent suppressor cells in the uterus of mated and pseudopregnant mice. *Journal of Reproductive Immunology*, **10**, 201–217.

Brodsky, W.Y. and Uryvaeva, I.V. (1977) Cell polyploidy: its relation to tissue growth and function. *International Review of Cytology*, **50**, 275–332.

Brody, S. (1969) *Foetus and Placenta* (eds A. Klopper and E. Diczfalusy), Blackwell Scientific Publications, Oxford pp. 299–411.

Brunette, M.G. (1988) Calcium transport through the placenta. *Canadian Journal of Physiology and Pharmacology*, **66**, 1261–1269.

Bryant Greenwood, G.D., Rees, M.C.P. and Turnbull, G.D. (1987) Immunohistochemical localisation of relaxin, prolactin and prostaglandin synthesase in human amnion, chorion and decidua. *Journal of Endocrinology*, **114**, 491–496.

Bryden, M.M., Evans, H.E. and Binns, W. (1972) Embryology of the sheep. I. Extra embryonic membranes and the development of body form. *Journal of Morphology*, **138**, 160–186.

Buckrell, B.C., Gartley, C.J., Mehren, K.G., Crawshaw, G.J., Johnson, W.H., Barker, I.K., Coghill, C., Challis, J.R.G. and Goodrowe, K.L. (1990) Failure to maintain interspecific pregnancy after transfer of Dall's sheep embryos to domestic ewes. *Journal of Reproduction and Fertility*, **90**, 387–394.

Bulmer, J.N., Billington, W.D. and Johnson, P.M. (1984) Immunohistological identification of trophoblast populations in early human pregnancy with the use of monoclonal antibodies. *American Journal of Obstetrics and Gynecology*, **148**, 19–26.

Bulmer, J.N., Morrison, L., Johnson, P.M. and Meager, A. (1990) Immunohistochemical localisation of interferons in human placental tissue in normal ectopic and molar pregnancy. *American Journal of Reproductive Immunology*, **22**, 109–116.

Bunch, T.D., Foote, W.C. and Spillett, J.J. (1976) Sheep–goat hybrid karyotypes. *Theriogenology*, **6**, 379–385.

Burd, L.I., Jones Jr., M.D., Simmons, M.A., Makowski, E.L., Meschia, G. and Battaglia, F.C. (1975) Placental production and foetal utilization of lactate and pyruvate. *Nature*, **254**, 710–711.

Burgess, T.L. and Kelly, R.B. (1987) Constitutive and regulated secretion of proteins. *Annual Review of Cell Biology*, **3**, 243–293.

Burke, J.L., Rice, G.E., Ralph, M.M. and Thorburn, G.D. (1989) Effects of calcium availability on the release of ovine choriomammotropin from cotyledonary cells incubated *in vitro*. *Comparative Biochemistry and Physiology*, **93A**, 489–492.

Burton, G.J. (1982) Review Article. Placental uptake of maternal erythrocytes: a comparative study. *Placenta*, **3**, 407–434.

Burton, G.J. (1987) The fine structure of the human placental villus as revealed by scanning electron microscopy. *Scanning Microscopy*, **1**, 1811–1828.

Burton, G.J. (1990) On the varied appearances of the human placenta villous surface visualised by scanning electron microscopy. *Scanning Microscopy*, **4**, 501–507.

Burton, G.J. and Tham, S.W. (1992) Formation of vasculosyncytial membrane in the human placenta. *Journal of Developmental Physiology*, **18**, 43–47.

Busch, L.C., Winterhager, E. and Fischer, B. (1986) Regeneration of the uterine epithelium in later stages of pseudopregnancy in the rabbit. An ultrastructural study. *Anatomy and Embryology*, **174**, 97–104.

Butler, H. (1967) The giant cell trophoblast of the Senegal galago (*Galago senegalensis*) and its bearing on the evolution of the primate placenta. *Journal of Zoology*, **152**, 195–207.

Byatt, J.C. and Schimonuta, K., Duello, T.M. and Bremel, R.D. (1986) Isolation and characterisation of multiple forms of bovine placental lactogen from secretory granules of the fetal cotyledon. *Endocrinology*, **119**, 1343–1350.

Byatt, J.C., Warren, W.C., Eppard, P.J., Staten, N.R., Krivi, G.G. and Collier, R.J. (1992) Ruminant placental lactogens: structure and the biology. *Journal of Animal Science*, **70**, 2911–2923.

Campbell, W.J., Deb, S., Kwok, S.C.M., Joslin, J.A. and Soares, M.J. (1989) Differential ex-

pression of placental lactogen II and prolactin like protein A in the rat placenta. *Endocrinology*, **125**, 1565–1574.

Cardell, R.R., Hisaw, F.L. and Dawson, A.B. (1969) The fine structure of granular cells in the uterine endometrium of the Rhesus monkey Macaca mulatta with a discussion of the possible function of these cells in relaxin secretion. *American Journal of Anatomy*, **124**, 307–340.

Carnegie, J.A., Chan, J.S.D., McCully, M.E., Robertson, H.A. and Friesen, H.G. (1982) The cellular localisation of chorionic somatomammotropin in ovine chorion. *Journal of Reproduction and Fertility*, **66**, 9–16.

Carpenter, S.J. (1972) Light and electron microscope observations on the morphology of the chorioallantoic placenta of the golden hamster (*Cricetusa* (*ureatus*)) Days 7 through 9 of gestation. *American Journal of Anatomy*, **135**, 445–476.

Carpenter, S.J. (1975) Ultrastructural observations on the maturation of the placental labyrinth of the Golden Hamster (Days 10–16 (term) of gestation). *American Journal of Anatomy*, **143**, 315–348.

Carpenter, S.J. (1982) Tropoblast invastion and alteration of maternal arteries in the pregnant hamster. *Placenta*, **3**, 219–242.

Carstensen, M., Leichtweiss, H.-P., Molsen, G. and Schröer, H. (1977) Evidence for a specific transport of D-hexoses across the human term placenta *in vitro*. *Archiv für Gynäkologie*, **222**, 187–196.

Carter, A.M. (1975) Placental circulation, in *Comparative Placentation: Essays in Structure and Function* (ed. D.H. Steven), Academic Press, New York, pp. 108–160.

Carter, A.M., Gothlin, J. and Olin, T. (1971) An angiographic study of the structure and function of uterine and maternal placental vasculature in the rabbit. *Journal of Reproduction and Fertility*, **25**, 201–210.

Casey, M.L., Winkel, C.A., Porter, J.C. and MacDonald, P.C. (1983) Endocrine regulation of the initiation of parturition. *Clinics in Perinatology*, **10**, 709–712.

Casida, L.E. and Warwick, E.J. (1945) The necessity of the corpus luteum for maintenance of pregnancy in the ewe. *Journal of Animal Science*, **4**, 34–36.

Castellucci, M., Scheper, M., Scheffen, I., Celora, A. and Kaufmann, P. (1990) The development of the human placental villous tree. *Anatomy and Embryology*, **181**, 117–128.

Cate-Hoedemaker, N.J. (1933) Beitrage zur kenntnis der plazentation bei Haien und Reptilien. *Zeitschrift Zellforschung & Mikroskopisches Anatomie*, **18**, 299–345.

Challis, J.R.G., Dilley, S.R., Robinson, J.S. and Thorburn, G.D. (1976) Prostaglandins in the circulation of the foetal lamb. *Prostaglandins*, **11**, 1041–1052.

Chan, J.S.D., Robertson, H.A. and Friesen, H.G. (1978) Maternal and fetal concentrations of OPL measured by radioimmunoassay. *Endocrinology*, **102**, 1606–1613.

Chan, J.S.D., Nie, Z.-R., Seidah, N.G. and Chretien, M. (1986) Inhibition of binding of OPL to GH and PRL receptors by monoclonal antibodies. *Endocrinology*, **119**, 2623–2628.

Chan, J.S.D., Nie, Z.-R. and Pang, S.C. (1990) Cellular localisation of ovine placental lactogen using monoclonal antibodies. *Animal Reproductive Science*, **23**, 33–40.

Chaouat, G. (1987) Placental immunoregulatory factors. *Journal of Reproductive Immunology*, **10**, 179–188.

Chatterjee, S., Hasrouni, S. and Lala, P.R. (1982) Localisation of paternal H2K antigens on murine trophoblast cells *in vivo*. *Journal of Experimental Medicine*, **155**, 1679–1689.

Chavez, D.J. and Enders, A.C. (1982) Lectin binding of mouse blastocysts: appearance of Dolichos biflorus binding sites on the trophoblast during delayed implantation and their subsequent disappearance during implantation. *Biology of Reproduction*, **26**, 545–552.

Chen, T.T., Bazer, F.W., Gebhardt, B.M. and Roberts, R.M. (1975) Uterine secretion in mammals. *Biology of Reproduction*, **13**, 304–313.

Chêne, N., Martal, J. and Charrier, J. (1988) Ovine chorionic somatomammotropin and fetal growth. *Reproduction Nutrition and Development*, **28**, 1707–1730.

Cherny, R.A. and Findlay, J.K. (1990) Separation and culture of endometrial epithelial and stromal cells: evidence of morphological and functional polarity. *Biology of Reproduction*, **43**, 241–250.

Christofferson, R.H. and Nillson, B.O. (1988) Morphology of the endometrial vasculature during early placentation in the rat. *Cell and Tissue Research*, **253**, 209–220.

Clark, D.A. (1985a) Maternofetal relations. *Immunology Letters*, **9**, 239–247.

Clark, D.A. (1985b) Prostaglandins and immunoregulation during pregnancy. *American Journal of Reproduction and Immunological Microbiology*, **9**, 111–112.

Clark, D.A., Croy, B.A., Wegmann, T.G. and Chaouat, G. (1987) Immunological and paraimmunological mechanisms in spontaneous abortion: recent insights and future directions. *Journal of Reproductive Immunology*, **12**, 1–12.

Clark, D.A., Slapsys, R., Croy, B.A., Krcek, J. and Rossant, J. (1984) Local active suppression by suppressor cells in the decidua: a review. *American Journal of Reproduction and Immunological Microbiology*, **5**, 78–83.

Clark, D.A., Slapsys, R., Chaput, A., Walker, C., Brierley, J., Daya, S. and Rosenthal, K.L. (1986) Immunoregulatory molecules of trophoblast and decidual suppressor cell origin at the maternofetal interface. *American Journal of Reproduction and Immunological Microbiology*, **10**, 100–104.

Clutton-Brock, T.H. (1991) *The Evolution of Parental Care: Monographs in Behavior and Ecology* (eds J.R. Krebs and T.H. Clutton-Brock), Princeton University Press, Princeton, New Jersey.

Coceani, F., Olley, P.M., Bisha, I., Bodach, E. and White, E.P. (1978) Significance of the prostaglandin system to the control of muscle tone of the ductus arteriosus. *Advances in Prostaglandin and Thromboxane Research*, **4**, 325–333.

Colbern, C.T. and Main, E.K. (1991) Immunology of the maternal placental interface in normal pregnancy. *Seminars in Perinatology*, **15**, 196–205.

Cole, W.C. and Garfield, R.E. (1989) Ultrastructure of the myometrium, in *Biology of the Uterus*, 2nd edn, Chapter 15 (eds R.M. Wynn and W.P. Jollie), Plenum, New York, pp. 455–504.

Colosi, P., Ogren, L., Thordarson, G. and Talamantes, F. (1987) Purification and partial characterisation of two prolactin like glycoprotein hormone complexes from the mid pregnant mouse conceptus. *Endocrinology*, **120**, 2500–2511.

Colosi, P., Thordarson, G., Hellmiss, R., Singh, K., Forsyth, I.A., Gluckman, P. and Wood, W.I. (1989) Cloning and expression of ovine placental lactogen. *Molecular Endocrinology*, **3**, 1462–1469.

Comline, R.S. and Silver, M. (1976) Some aspects of foetal and utero-placental metabolism in cows with indwelling vascular catheters. *Journal of Physiology, London*, **260**, 571–586.

Concannon, P.W., McCann, J.P. and Hemp, E.M. (1989) Biology and endocrinology of ovulation pregnancy and parturition in the dog. *Journal of Reproduction and Fertility*, Suppl. 39, 3–25.

Contractor, S.F. (1983) Metabolic and enzymatic activity of the human trophoblast, in *Biology of Trophoblast* (eds C. Loke and A. Whyte), Elsevier, Amsterdam, pp. 236–282.

Covone, A.E., Johnson, P.M., Mutton, D. and Adinolfi, M. (1984) Trophoblast cells in peripheral blood from pregnant women. *Lancet*, **ii**, 841–843.

Cowie, A.T., Daniel, P.M., Prichard, M.M.L. and Tindal, J.S. (1963) Hypophysectomy in pregnant goats and section of the pituitary stalk in pregnant goats and sheep. *Journal of Endocrinology*, **28**, 93–102.

Crawford, R.J., Tregear, G.W. and Niall, H.D. (1986) The nucleotide sequences of baboon chorionic gonadotropin β-subunit genes have diverged from the human. *Gene*, **46**, 161–169.

Crepeau, M.A., Yamashito, S. and Croy, B.A. (1989) Morphological demonstration of the failure of Mus caroli trophoblast in the Mus musculus uterus. *Journal of Reproduction and Fertility*, **86**, 277–288.

Crews, D. (1992) Chemical communication – The language of pheromones. *Nature*, **359**, 492.

Critchley, G.R. and Burton, G.J. (1987) Intralobular variation in barrier thickness in the mature human placenta. *Placenta*, **8**, 185–194.

Crombie, P.R. (1972) The morphology and ultrastructure of the pig's placenta throughout pregnancy. PhD thesis, University of Cambridge.

Cross, J.C. and Roberts, R.M. (1989) Porcine conceptuses secrete an interferon during the preattachment period of early pregnancy. *Biology of Reproduction*, **40**, 1109–1118.

Croy, B.A. and Chapeau, C. (1990) Evaluation of pregnancy immunotrophism hypothesis by assessment of the reproductive performance of young adult mice of genotype scid.scid bg/bg. *Journal of Reproduction and Fertility*, **88**, 231–239.

Croy, B.A. and Kassorf, S.A. (1989) Evaluation of the murine metrial gland for immunological function. *Journal of Reproductive Immunology*, **15**, 51–70.

Croy, B.A., Rossant, J. and Clark, D.A. (1982) Histological and immunological studies of postimplantation death of *Mus caroli* embryos in the *Mus musculus* uterus. *Journal of Reproduction and Immunology*, **4**, 277–293.

Croy, B.A., Gambel, P., Rossant, J. and Wegmann, T.G. (1985) Characterisation of murine decidual NK cells and their relevance to

the success of pregnancy. *Cellular Immunology*, **93**, 315–326.

Crump, A., Donaldson, W.L., Miller, J., Kydd, J.H., Allen, W.R. and Antczak, D. F. (1987) Expression of MHC antigens on horse trophoblast. *Journal of Reproduction and Fertility*, Suppl. 35, 379–388.

Csapo, A.I. (1977) The 'see-saw' theory of parturition, in *The Fetus and Birth* (eds J. Knight and M. O'Connor), Ciba Foundation Symposium, No. 47 (New Series), Elsevier, Amsterdam, pp. 159–195.

Csapo, A.I. and Corner, G.W. (1952) The antagonistic effects of estrogen and progesterone on the staircase phenomenon in uterine muscle. *Endocrinology*, **51**, 378–385.

Cukierski, M.A. (1987) Synthesis and transport studies of the intrasyncytial lamina: an unusual placental basement membrane in the little brown bat Myotis lucifugus. *American Journal of Anatomy*, **178**, 387–409.

Currie, W.B., Card, C.E., Michel, F.J. and Ignotz, G. (1990) Purification, partial characterisation and development of a specific RIA for goat placental lactogen. *Journal of Reproduction and Fertility*, **90**, 25–36.

Dallenbach-Hellweg, G. (1967) Endometrial granulocytes and implantation. *Excerpta Medica International Congress*, Series 133, 411–418.

Dallenbach-Hellweg, G. (1981) *Histopathology of the Endometrium*, Springer, Berlin.

Dantzer, V. (1985) Electron microscopy of the initial stages of placentation in the pig. *Anatomy and Embryology*, **172**, 281–293.

Dantzer, V., Bjorkman, N. and Hasselager, E. (1981) An electron microscopic study of histotrophe in the interareolar part of the porcine placenta. *Placenta*, **21**, 19–28.

Davies, J. and Davenport, G.R. (1979) Symplasma formation and decidualisation in the pseudopregnant rabbit after intraluminal instillation of tricaprylin. *Journal of Reproduction and Fertility*, **55**, 141–145.

Davies, J. and Glasser, S.R. (1968) Histological and fine structural observations on the placenta of the rat. I. Organisation of the normal placenta. II. Changes after surgical removal of the fetus. *Acta Anatomica*, **69**, 542–608.

Davies, J. and Hoffman, L.H. (1975) Studies on the progestational endometrium of the rabbit. II. Electron microscopy day 0 to day 13 of gonadotrophin induced pseudopregnancy. *American Journal of Anatomy*, **142**, 335–366.

Davies, J., Dempsey, E.W. and Amoroso, E.C. (1961a) The subplacenta of the guinea pig: an electronmicroscope study. *Journal of Anatomy*, **95**, 311–324.

Davies, J., Dempsey, E.W. and Amoroso, E.C. (1961b) The subplacenta of the guinea pig: development, histology and histochemistry. *Journal of Anatomy*, **95**, 457–473.

Dawes, G.S. (1968) *Foetal and neonatal physiology: a comparative study of the changes at birth*. Year Book Medical Publishers, Chicago.

Daya, S., Clark, D.A., Derlin, C. and Javrell, J. (1985) Preliminary characterisation of two types of suppressor cells in the human uterus. *Fertility and Sterility*, **44**, 778–785.

De, M., Sandford, T.R. and Wood, G.W. (1993) Expression of IL1, 6 and TNFα in the mouse uterus during the peri-implantation period and pregnancy. *Journal of Reproduction and Fertility*, **97**, 83–89.

Deane, H.W., Rubin, B.L., Driks, E.C., Lobel, B.L. and Leipsner, G. (1962) Trophoblast giant cells in placentas of rats and mice and their probable role in steroid hormone production. *Endocrinology*, **70**, 407–419.

Del Pino, E.M., Galarza, M.L., DeAlbuja, C.M. and Humphries, A.A. (1975) The maternal pouch and development in the marsupial frog Gastrotheca riobambae. *Biological Bulletin*, **149**, 480–491.

Dempsey, E.W. (1969) Comparative aspects of the placentae of certain African mammals. *Journal of Reproduction and Fertility*, **6**, 189–192.

Denamur, R. and Martinet, J. (1955) Effets de l'ovariectomie chez la brebis pendant la gestation. *Comptes Rendus des Seances Societe de Biologie*, Paris **149**, 2105–2107.

Denker, H.W. (1978) Role of trophoblastic factors in implantation, in *Novel Aspects of Reproductive Physiology* (eds C.H. Spilman and J.W. Wilks), Spectrum, New York. 181–182.

Denker, H.W. (1993) Implantation: a cell biological paradox. *Journal of Experimental Zoology*, **266**, 541–558.

Denker, H.W. and Tyndale-Biscoe, C.H. (1986) Embryonic implantation and proteinase activity in a marsupial (*Macropus eugenii*) histochemical patterns. *Cell and Tissue Research*, **246**, 279–291.

Denker, H.W., Eng, L.W. and Hamner, C.E. (1978) Studies on the early development and implantation in the cat. II. Implantation processes. *Anatomy and Embryology*, **54**, 39–54.

Diczfalusy, E. and Trën, P. (1961) Endocrine func-

tions of the human placenta. *Vitamins and Hormones*, **19**, 229–250.

Dobashi, K., Ajika, K., Kambegawa, A. and Arai, K. (1985) Localisation and distribution of unconjugated steroid hormones in the normal placenta at term. *Placenta*, **6**, 445–454.

Donaldson, W.L., Zhang, G.H., Oriol, J.G. and Antczak, D.F. (1990) Invasive equine trophoblast expresses conventional class I MHC antigens. *Development*, **110**, 63–71.

Donaldson, W.L., Oriol, J.G., Plavin, A. and Antczak, D.F. (1992) Developmental regulation of class I MHC antigen expression by equine trophoblast cells. *Differentiation*, **52**, 69–78.

Dracopoli, N.C., Rettig, W.J., Whitfield, G.K., Darlington, G.R., Spengler, B.A., Biedler, J.L., Old, L.J. and Kouridea, I.A. (1986) Assignment of the gene for the β subunit of thyroid-stimulating hormone to the short arm of human chromosome 1. *Proceedings of the National Academy of Sciences of the USA*, **83**, 1822–1826.

Dresser, B.L., Pope, C.E., Kramer, L., Kuehn, G., Dahlhassen, R.D., Maruska,E.J., Reece, B. and Thomas, W.D. (1985) Birth of bongo antelope (*Tragelaphus euryceros*) to eland antelope (*Tragelaphus oryx*) and cryopreservation of bongo embryos (abstract). *Theriogenology*, **23**, 190.

Drieux, H. and Thiery, G. (1951) La placentation chez Mammifères domestiques. III. Placenta des Bovides. *Recueil de Médecin Vétérinaire*, **127**, 5–25.

Drummond-Robinson, G. and Asdell, S.A. (1926) The relation between the corpus luteum and the mammary gland. *Journal of Physiology, London*, **61**, 608–614.

Duckworth, M.L., Schroedter, I.C. and Friesen, H.G. (1990) Cellular localisation of rat placental lactogen II and rat prolactin like proteins A and B by in situ hybridisation. *Placenta*, **11**, 143–155.

Duello, T.M., Byatt, J.C. and Bremel, R.D. (1986) Immunohistochemical localisation of placental lactogen in binucleate cells of bovine placentomes. *Endocrinology*, **119**, 1351–1355.

Du Mesnil Du Buisson, F. and Dauzier, L. (1957) Influence de l'ovariectomie chez la Tunie pendant la gestation. *Comptes Rendus des Séances Sociétié de Biologie*, Paris, **151**, 311–313.

Durrant, B. and Benirschke, K. (1981) Embryo transfer in exotic animals. *Theriogenology*, **15**, 77–83.

Dziuk, P.J., Polge, C. and Rowson, L.E. (1964) Intrauterine migration and mixing of embryos in swine following egg transfer. *Journal of Animal Science*, **23**, 37–42.

Edson, J.L., Hudson, D.G. and Hull, D. (1975) Evidence for increased fatty acid transfer across the placenta during a maternal fast in rabbits. *Biology of the Neonate*, **27**, 50–55.

Edwards, D., Jones, C.J.P., Sibley, C.P. and Nelson, D.M. (1993) Paracellular permeability pathways in the human placenta: a quantitative and morphometric study of maternofetal transfer of HRP. *Placenta*, **14**, 63–73.

Elcock, J.M. and Searle, R.F. (1985) Antigen presenting capacity of mouse decidual tissue and placenta. *American Journal of Reproductive Immunology*, **7**, 99–103.

Ellington, S.K.L. (1985) A morphological study of the development of the allantois of rat embryos *in vivo*. *Journal of Anatomy*, **142**, 1–11.

Ellington, S.K.L. (1987) A morphological study of the development of the chorion of rat embryos. *Journal of Anatomy*, **150**, 247–263.

Elphick, M.C., Hull, D. and Broughton-Pipkin, F. (1979) The transfer of fatty acids across the sheep placenta. *Journal of Developmental Physiology*, **1**, 31–45.

Emane, M.N., Delouis, C., Kelly, P.A. and Djiane, J. (1986) Evolution of PRL and PL receptors in ewes during pregnancy and lactation. *Endocrinology*, **118**, 695–704.

Enders, A.C. (1965) A comparative study of the fine structure of the trophoblast in hemochorial placentas. *American Journal of Anatomy*, **116**, 29–68.

Enders, A.C. (1982) Whither studies of placental morphology. *Journal of Reproduction and Fertility*, **31**, 9–15.

Enders, A.C. (1989) Trophoblast differentiation during the transition from trophoblast plate to lacunar stage of implantation in the rhesus monkey and human. *American Journal of Anatomy*, **186**, 85–98.

Enders, A.C. (1991) Structural responses of the primate endometrium to implantation. *Placenta*, **12**, 309–325.

Enders, A.C. and Enders, R.K. (1969) The placenta of the four eyed opossum (*Philander opossum*). *Anatomical Record*, **165**, 431–450.

Enders, A.C. and Given, R.L. (1977) The endometrium of delayed and early implantation, in *Biology of the Uterus* (ed. R.M. Wynn), Plenum, New York, 203–238.

Enders, A.C. and King, B.F. (1991) Early stages of trophoblast invasion of the maternal vascular system during implantation in the macaque and

baboon. *American Journal of Anatomy*, **192**, 329–346.

Enders, A.C. and Liu, I.K.M. (1991a) Lodgement of the equine blastocyst in the uterus from fixation through endometrial cup formation. *Journal of Reproduction and Fertility*, Suppl. 44, 427–438.

Enders, A.C. and Liu, I.K.M. (1991b) Trophoblast–uterine interactions during equine chorionic girdle cell maturation migration and transformation. *American Journal of Anatomy*, **192**, 366–381.

Enders, A.C. and Schlafke, S. (1969) Cytological aspects of trophoblast–uterine interaction in early implantation. *American Journal of Anatomy*, **125**, 1–30.

Enders, A.C. and Schlafke, S. (1971a) Implantation in the ferret: epithelial penetration. *American Journal of Anatomy*, 291–316.

Enders, A.C. and Schlafke, S. (1971b) Penetration of the uterine epithelium during implantation in the rabbit. *American Journal of Anatomy*, **132**, 219–240.

Enders, A.C. and Welsh, A.O. (1993) Structural interactions of trophoblast and uterus during hemochorial placenta formation. *Journal of Experimental Zoology*, **266**, 578–587.

Enders, A.C. and Wimsatt, W.A. (1968) Formation and structure of the hemodichorial chorioallantoic placenta of the bat (*Myotis lucifugus* Inc.). *American Journal of Anatomy*, **122**, 453–463.

Enders, A.C. and Wimsatt, W.A. (1971) Transport and barrier function in the chorioallantoic placenta of the bat, *Myotis lucifugus*. *Anatomical Record*, **170**, 381–400.

Enders, A.C., Schlafke, S. and Welsh, A.O. (1980) Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in the rat. *American Journal of Anatomy*, **159**, 59–72.

Enders, A.C., Welsh, A.O. and Schlafke, S. (1985) Implantation in the rhesus monkey: endometrial responses. *American Journal of Anatomy*, **173**, 147–169.

Faria, T.N., Deb, S., Kwok, S.C.M., Talamantes, F. and Soares, M.J. (1990) Ontogeny of placental lactogens and placental lactogen II expression in the developing rat placenta. *Developmental Biology*, **141**, 279–291.

Farin, C.E., Imakawa, K. and Roberts, R.M. (1989) In situ localisation of mRNA for the IFN OTP-1 during early embryonic development in the sheep. *Molecular Endocrinology*, **3**, 1099–1107.

Farin, C.E., Imakawa, K., Hansen, T.R., McDonnell, J.J., Murphy, C.N., Farin, P.W. and Roberts, R.M. (1990) Expression of trophoblast interferon genes in sheep and cattle. *Biology of Reproduction*, **43**, 210–218.

Farkash, Y., Timberg, R. and Orly, J. (1986) Preparation of an antiserum to rat cytochrome P450 cholesterol side chain cleavage enzyme and its use for ultrastructural localisation of the immunoreactive enzyme by protein A gold technique. *Endocrinology*, **118**, 1353–1365.

Fatemi, S.H. (1987) The role of secretory granules in the transport of basement membrane components: ARG studies of rat parietal yolk sac employing ^{3}H proline as a precursor of type IV collagen. *Connective Tissue Research*, **16**, 1–14.

Faulk, W.P. and Hsi, B.L. (1983) Immunobiology of human trophoblast membrane antigens, in *Biology of Trophoblast*, (eds C. Loke and A. Whyte), Elsevier, Amsterdam, pp. 535–565.

Faulk, W.P. and McIntyre, J.A. (1983) Immunological studies of human trophoblast: markers, subsets and functions. *Immunological Reviews*, **75**, 139–175.

Fehilly, C.B., Willadsen, S.M. and Tucker, E.M. (1984) Interspecific chimaerism between sheep and goat. *Nature*, **307**, 634–636.

Ferguson, M.M. and Christie, G.A. (1967) Distribution of hydroxysteroid dehydrogenase in the placental and foetal membranes of various mammals. *Journal of Endocrinology*, **38**, 291–306.

Ferry, B.L., Starkey, P.M., Sargent, I.L., Watt, G.M.O., Jackson, M. and Redman, C.W.G. (1990) Cell populations in the human early pregnancy decidua: NK activity and response to interleukin 2 of CD56 positive large granular lymphocytes. *Immunology*, **70**, 446–452.

Fincher, K.B., Bazer, F.W., Hansen, P.J., Thatcher, W.W. and Roberts, R.M. (1986) Proteins secreted by the sheep conceptus suppress induction of uterine $PGF_{2\alpha}$ release by oestradiol and oxytocin. *Journal of Reproduction and Fertility*, **76**, 425–433.

Finn, C.A. (1986) Implantation, menstruation and inflammation. *Biological Reviews*, **61**, 313–328.

Finn, C.A. and Bredl, J.C.S. (1973) Studies on the development of the implantation reaction in the mouse uterus: Influence of actinomycin D. *Journal of Reproduction and Fertility*, **34**, 247–253.

Finn, C.A. and Porter, D.G. (1975) The Uterus, *Handbooks in Reproductive Biology*, Vol. 1, Elek Science, London.

First, N.L. and Bosc, M.J. (1979) Proposed mechanisms controlling parturition and the induction

of parturition in swine. *Journal of Animal Science*, **48**, 1407–1421.

First, N.L. and Staigmiller, R.B. (1973) Effects of ovariectomy, dexamethasone and progesterone on the maintenance of pregnancy in swine. *Journal of Animal Science*, **37**, 1191–1194.

Firth, J.A., Sibley, C.P. and Ward, B.S. (1986a) Histochemical localisation of phosphatases in the pig placenta. I. Nonspecific phosphatases and their relation to uteroferrin. *Placenta*, **7**, 17–25.

Firth, J.A., Sibley, C.P. and Ward, B.S. (1986b) Histochemical localisation of phosphatases in the pig placenta. II. Potassium dependent and potassium independent paranitrophenylphosphatases at high pH: relationship to sodium dependent ATP'ase. *Placenta*, **7**, 26–36.

Fisher, D.A. (1986) The unique endocrine milieu of the fetus. *Journal of Clinical Investigation*, **78**, 603–611.

Fischer, T.V. (1971) Placentation in the American beaver (*Castor canadensis*). *American Journal of Anatomy*, **131**, 159–184.

Fischer, T.V. and Mossman, H.W. (1969) The fetal membranes of Pedetes capensis and their taxonomic significance. *American Journal of Anatomy*, **124**, 89–116.

Flint, A.P.F. (1983) Controlling placental endocrine function in domestic animals. *Current Topics in Experimental Endocrinology*, **5**, 75–95.

Flint, A.P.F. and Burton, R.D. (1984) Properties and ontogeny of the glucocorticoid receptor in the placenta and fetal lung of the sheep. *Journal of Endocrinology*, **103**, 31–42.

Flint, A.P.F., Kingston, E.J., Robinson, J.S. and Thorburn, G.D. (1978) Initiation of parturition in the goat: evidence for control by foetal glucocorticoid through activation of placental C_{21}-steroid 17α-hydroxylase. *Journal of Endocrinology*, **78**, 367–378.

Flint, A.P.F., Henville, A. and Christie, W.B. (1979) Presence of placental lactogen in bovine conceptuses before attachment. *Journal of Reproduction and Fertility*, **56**, 305–308.

Flint, A.P.F., Burton, R.D. and Heap, R.B. (1983) Sources of progesterone during gestation in Barbary sheep (*Ammotragus lervia*). *Journal of Endocrinology*, **98**, 283–288.

Folkart, G.R., Dancis, J. and Money, W.L. (1960) Transfer of carbohydrates across guinea-pig placenta. *American Journal of Obstetrics and Gynecology*, **80**, 221–223.

Ford, S.P., Weber, L.J. and Stormshak, F. (1977) Role of estradiol-17β and progesterone in regulating constriction of ovine uterine arteries. *Biology of Reproduction*, **17**, 480–483.

Forsyth, I.A. (1986) Variation among species in the endocrine control of mammary gland growth and function: Structure of PRL, GH and PL. *Journal of Dairy Science*, **69**, 886–903.

Forsyth, I.A. (1991) The biology of the placental PRL/GH family. *Oxford Reviews of Reproductive Biology*, **13**, 97–148.

Fraser, E.A. and Renton, R.M. (1940) Observations on the breeding and development of the viviparous fish Heterandria formosa. *Quarterly Journal of Microscopical Science*, **81**, 479–520.

Freemark, M. and Handwerger, S. (1986) The glycogenic effects of PL and GH in ovine fetal liver are mediated through binding to specific fetal OPL receptors. *Endocrinology*, **118**, 613–618.

Freemark, M., Cromer, M., Korner, G. and Handwerger, S. (1987) A unique placental lactogen receptor: implications for fetal growth. *Endocrinology*, **120**, 1865–1872.

Friess, A.E., Sinowatz, F., Skolek-Winnischen, R. and Trautner, W. (1981) The placenta of the pig. II. the ultrastructure of the areolae. *Anatomy and Embryology*, **163**, 43–53.

Fuchs, A.R., Helmer, H., Chang, S.M. and Fields, M.J. (1992) Concentration of OT receptors in the placenta and fetal membranes of cows during pregnancy and labour. *Journal of Reproduction and Fertility*, **96**, 775–783.

Fujimoto, S., Hamasaki, K., Ueda, H. and Kagawa, H. (1986) Immunoelectronmicroscope observations on secretion of HPL in the human chorionic villi. *Anatomical Record*, **216**, 68–72.

Galton, M. (1962) DNA content of placental nuclei. *Journal of Cell Biology*, **13**, 183–191.

Gambel, P., Rossant, J., Hunziker, R.D. and Wegmann, T. (1985) Origin of decidual cells in murine pregnancy and pseudopregnancy. *Transplantation*, **39**, 443–445.

Gardner, R. (1975) Origins and properties of trophoblast, in *Immunology of Trophoblast* (eds. R.G. Edwards, C.W.S. Howe and M.H. Johnson), Cambridge University Press, Cambridge, pp. 43–61.

Garfield, R.F., Robideau, S., Challis, J.R.G. and Daniel, E.E. (1979) Hormonal control of gap junction formation in sheep myometrium during parturition. *Biology of Reproduction*, **21**, 999–1007.

Garfield, R.E., Blennerhassett, M.G. and Miller,

S.M. (1988) Control of myometrial contractility: role and regulation of gap junctions. *Oxford Review of Reproductive Biology*, **10**, 436–490.

Geier, G., Schuhmann, R. and Kraus, H. (1975) Regionale unterschiedliche Zellproliferation innerhalb der Plazentome reifer menschlicher Plazenten. *Archiv Gynakologie*, **218**, 31–37.

Geisert, R.D., Renegar, R.H., Thatcher, W.W., Roberts, R.M. and Bazer, F.W. (1982a) Establishment of pregnancy in the pig. I. Interrelationship between pre-implantation development of the pig blastocyst and uterine endometrial secretions. *Biology of Reproduction*, **27**, 925–939.

Geisert, R.D., Brookbank, J.W., Roberts, R.M. and Bazer, F.W. (1982b) Establishment of pregnancy in the pig. II. Cellular remodelling of the porcine blastocyst during elongation on day 12 of pregnancy. *Biology of Reproduction*, **27**, 941–955.

Geisert, R.D., Thatcher, W.W., Robert, R.M. and Bazer, F.W. (1982c) Establishment of pregnancy in the pig. III. Endometrial secretory response to estradiol administration on day 11 of the estrous cycle. *Biology of Reproduction*, **27**, 957–965.

Geisert, R.D., Lee, C.L., Simmen, F.A., Zavi, M.T., Fliss, A.E., Bazer, F.W. and Simmen, R.C.M. (1991) Expression of mRNAs encoding IGFI and II and IGFBP2 in bovine endometrium during the estrus cycle and early pregnancy. *Biology of Reproduction*, **45**, 975–983.

Gibori, G., Ralison, B., Basuray, R., Rowe, M.C., Hunzicker, B. and Dunn, M. (1984) Endocrine role of the decidual tissue: Decidual and luteotrophic regulation of luteal and cyclase activity, luteal hormone receptors and steroidogenesis. *Endocrinology*, **115**, 1153–1157.

Gill, T.A. and Wegmann, T.G. (1987) *Immunoregulation and Fetal Survival*, Oxford University Press.

Ginther, O.J. (1979) *Reproductive Biology of the Mare* (privately published book). 4343 Garfoot Rd Cross Plains, Wisconsin 53528, USA.

Ginther, O.J. (1983) Mobility of early equine conceptus. *Theriogenology*, **19**, 603–610.

Ginther, O.J. (1984a) Mobility of twin embryonic vesicles in mares. *Theriogenology*, **22**, 83–95.

Ginther, O.J. (1984b) Intrauterine movement of the early conceptus in barren and postpartum mares. *Theriogenology*, **21**, 633–644.

Ginther, O.J. (1989) The nature of embryo reduction in mares with twin conceptuses: deprivation hypothesis. *American Journal of Veterinary Research*, **50**, 45–52.

Given, R.L. and Enders, A.C. (1989) The endometrium of delayed and early implantation, in *Biology of the Uterus*, 2nd edn, Chapter 8 (eds R.M. Wynn and W.P. Jollie), Plenum Medical, New York, pp. 175–231.

Glasser, S.R. (1990) Biochemical and structural changes in uterine endometrial cell types following natural or artificial deciduogenic stimuli. *Trophoblast Research*, **4**, 377–416.

Glasser, S.R. and McCormack, S.A. (1980) Functional development of rat trophoblast and decidual cells during establishment of the hemochorial placenta. *Advances in Bioscience*, **25**, 165–198.

Gluckman, P.D., Breier, B.H. and Davis, J.R. (1987) Physiology of the somatotropic axis with particular reference to the ruminant. *Journal of Dairy Science*, **70**, 442–466.

Godkin, J.D., Bazer, F.W. and Roberts, R.M. (1984a) Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein synthesis. *Endocrinology*, **114**, 120–129.

Godkin, J.D., Bazer, F.W., Thatcher, W.W. and Roberts, R.M. (1984b) Proteins released by cultured day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. *Journal of Reproduction and Fertility*, **71**, 57–64.

Goldstein, S.R. (1926) A note on the vascular relations and areolae in the placenta of the pig. *Anatomical Record*, **34**, 25–33.

Gopalakrishna, A. and Karim, K.B. (1979) Fetal membranes and placentation in Chiroptera. *Journal of Reproduction and Fertility*, **56**, 417–429.

Gosseye, S. and Fox, H. (1984) An immunohistological comparison of the secretory capacity of villous and extravillous trophoblast in the human placenta. *Placenta*, **5**, 329–348.

Grandis, A.S. and Handwerger, S. (1983) Differential effects of ornithine on placental lactogen and GH secretion. *Journal of Endocrinology*, **97**, 175–178.

Gray, A.P. (1971) *Mammalian Hybrids*. Commonwealth Agricultural Bureau, Farnham Royal, Slough, UK, pp. 262.

Greenstein, J.S., Murray, R.W. and Foley, R.C. (1958) Observations on the morphogenesis and histochemistry of the bovine preattachment placenta between 16 and 33 days of gestation. *Anatomical Record*, **132**, 321–325.

Greiss, F.C. (1966) Pressure–flow relationship in

the gravid uterine vascular bed. *American Journal of Obstetrics and Gynecology*, **96**, 41–47.

Greiss, F.C. and Anderson, S.G. (1970) Effect of ovarian hormones on the uterine vascular bed. *American Journal of Obstetrics and Gynecology*, **107**, 829–836.

Gresham, E.L., James, E.J., Raye, J.R., Battaglia, F.C., Makowski, E.L. and Meschia,G. (1972) Production and excretion of urea by the foetal lamb. *Pediatrics*, **50**, 372–379.

Gross, T.S., Williams, W.F. and Russek-Cohen, E. (1991) Cellular changes in the peripartum bovine fetal placenta related to placental separation. *Placenta*, **12**, 27–35.

Grove, B.D. and Wourms, J.P. (1991) The follicular placenta of the viviparous fish Heterandria formosa. 1. Ultrastructure and development of the embryonic absorptive surface. *Journal of Morphology*, **209**, 265–284.

Gruenwald, P. (1973) Lobular structure of hemochorial primate placentas and its relationship to maternal vessels. *American Journal of Anatomy*, **136**, 133–152.

Grunert, E. (1986) Etiology and pathology of retained bovine placenta, in *Current Therapy in Theriogenology* (ed. D.A. Morrow) W.B. Saunders, London, **2**, pp. 237–242.

Guillette, L.J. (1981) On the occurrence of oviparous and viviparous forms of the Mexican lizard *Sceloporus aeneus*. *Herpetologica*, **37**, 11–15.

Guillette, L.J. (1982) The evolution of viviparity and placentation in the high elevation Mexican lizard *Sceloporus aeneus*. *Herpetologica*, **38**, 94–103.

Guillette, L.J., Lavia, L.A., Walker, N.J. and Roberts, D.K. (1984) Luteolysis induced by prostaglandin $F_{2\alpha}$ in the lizard, *Anolis carolinensis*. *General and Comparative Endocrinology*, **56**, 271–277.

Guillette, L.J., Herman, C.A. and Dickey, D.A. (1988) Synthesis of prostaglandin by tissues of the viviparous lizard, *Sceloporus aeneus*. *Journal of Herpetology*, **22**, 180–185.

Guillette, L.J. (1991) The evolution of viviparity in aminote vertebrates – new insights, new questions. *Journal of Zoology*, **223**, 521–526.

Guillomot, M. and Guay, P. (1982) Ultrastructural features of the cell surfaces of uterine and trophoblastic epithelium during embryonic attachment in the cow. *Anatomical Record*, **204**, 315–322.

Guillomot, M., Flechon, E., Wintenberger-Torres. S. (1981) Conceptus attachment in the ewe: an ultratructural study. *Placenta*, **2**, 169–182.

Guillomot, M., Flechon, E., Wintenberger-Torres. S. (1982) Cytochemical studies of the uterus and trophoblastic surface during blastocyst attachment in the ewe. *Journal of Reproduction and Fertility*, **65**, 1–8.

Guillomot, M., Michel, C., Gaye, P., Charlier, N., Trojan, J. and Martal, J. (1990) Cellular localisation of an embryonic interferon, ovine trophoblastin, and its mRNA in sheep embryos during early pregnancy. *Biologie Cellulaire*, **68**, 205–211.

Habashi, S., Burton, G.J. and Steven, D.H. (1983) Morphological study of the fetal vasculature of the human term placenta: scanning electron microscopy of corrosion casts. *Placenta*, **4**, 41–56.

Hafez, E.S.E. and Tsutsumi, Y. (1966) Changes in endometrial vascularity during implantation and pregnancy in the rabbit. *American Journal of Anatomy*, **118**, 249–282.

Hagan, H. (1951) *Embryology of the Viviparous Insects*. Ronald Press, New York.

Hall, J. and Talamantes, F. (1984) Immunogold localisation of mouse placental lactogen in the mouse placenta. *Journal of Histochemistry and Cytochemistry*, **32**, 379–382.

Haluska, G.J. and Currie, W.B. (1988) Variation in plasma concentrations of oestradiol 17β and their relationship to those of progesterone, $PGF_{2\alpha}$and oxytocin across pregnancy and at parturition in pony mares. *Journal of Reproduction and Fertility*, **84**, 635–644.

Hamilton, D.W., Allen, W.R. and Moor, R.M. (1973) Origin of endometrial cups. III. Light and electron microscopic study of fully developed equine endometrial cups. *Anatomical Record*, **177**, 503–518.

Hamilton, W.J., Harrison, R.J. and Young, B.A. (1960) Aspects of placentation in certain Cervidae. *Journal of Anatomy*, **94**, 1–33.

Hamlett, W.C. (1989) Evolution and morphology of the placenta in sharks. *Journal of Experimental Zoology*, Suppl. 2, 35–52.

Hamlett, W.C. and Wourms, J.P. (1984) Ultrastructure of the preimplantation shark yolk sac placenta. *Tissue and Cell Research*, **16**, 613–625.

Hamlett, W.C., Wourms, J.P. and Hudson, J.S. (1985) Ultrastructure of the full term shark yolk sac placenta. I. Morphology and cell transport at the fetal attachment site. *Journal of Ultrastructure Research*, **91**, 192–206.

Hamlett, W.C., Eulitt, A.M., Jarrell, R.L. and Kelly, M.A. (1993) Utero-gestation and placen-

tation in Elasmobranchs. *Journal of Experimental Biology*, **266**, 347–367.

Hamon, M., Heap, R.B., Morgan, G., Wango, E. and Wooding, F.B.P. (1985) Steroid production by isolated of binucleate cells from ruminant placenta. *Journal of Physiology*, **371**, 218P.

Handwerger, S. (1991) The physiology of placental lactogen in human pregnancy. *Endocrine Reviews*, **12**, 329–339.

Hansen, P.J., Anthony, R.V., Bazer, F.W., Baumbach, G.A. and Roberts, R.M. (1985) *In vitro* synthesis and secretion of OTP-1 during the period of maternal recognition of pregnancy. *Endocrinology*, **117**, 1424–1430.

Harding, J.E., Jones, C.T. and Robinson, J.S. (1985) Studies on experimental growth retardation in sheep. The effects of a small placenta in restricting transport to and growth of the fetus. *Journal of Developmental Physiology*, **7**, 427–442.

Hasselager, E. (1985) Surface exchange area of the porcine placenta: morphometry of anisotropic interdigitating microvilli. *Journal of Microscopy*, **141**, 91–100.

Head, J.R. and Seelig, L.L. (1984) Lymphatic vessels in the uterine endometrium of virgin rats. *Journal of Reproductive Immunology*, **6**, 157–166.

Heap, R.B., Flint, A.P.F. and Staples, L.D. (1983) Endocrinology of trophoblast in farm animals, in *Biology of Trophoblast* (eds C. Loke and A. Whyte), Elsevier, Amsterdam, pp. 353–400.

Heap, R.B., Fleet, I.R. and Hamon, M. (1985) $PGF_{2\alpha}$ is transferred from the uterus to the ovary in the sheep by lymphatic and blood vascular pathways. *Journal of Reproduction and Fertility*, **74**, 645–656.

Hees, H., Moll, W., Wrobel, K.H and Hees, Z. (1987) Pregnancy induced structural changes and trophoblast invasion in the segmental mesometrial arteries of the guinea pig. *Placenta*, **8**, 609–626.

Helmer, S.D., Hansen, P.J., Anthony, R.V., Thatcher, W.W., Bazer, F.W. and Roberts,R.M. (1987) Identification of bovine trophoblast protein 1, a secretory protein immunologically related to OTP-1. *Journal of Reproduction*, **79**, 83–91.

Hemmings, W.A. (1957) Protein selection in yolk sac splanchnopleur of the rabbit: the total uptake as estimated as loss from the uterus. *Proceedings of the Royal Society*, **B 148**, 76–83.

Hill, D.J., Clemmons, D.R., Riley, S.C., Bassett, N. and Challis, J.R.G. (1993) Immunohistochemical localisation of IGFs and IGF1/2/3 binding proteins in the human placenta and fetal membranes. *Placenta*, **14**, 1–12.

Hill. J.P. (1932) The developmental history of the primates. *Philosophical Transactions of the Royal Society*, **221**, 45–178.

Hobson, B.M. and Wide, L. (1972) A comparison between chorionic gonadotrophins extracted from human, rhesus monkey and marmoset placentas. *Journal of Endocrinology*, **55**, 363–368.

Hodgen, G.D., Tullner, W.W., Vaitukaitis, J.L., Ward, D.N. and Ross, G.T. (1974) Specific radioimmunoassay of chorionic gonadotrophin during implantation in Rhesus monkeys. *Journal of Clinical Endocrinology and Medicine*, **39**, 457–464.

Hofmann, G.E., Scott, R.T., Bergh, P.A. and Deligdisch, L. (1991) Immunohistochemical localisation of EGF in human endometrium decidua and placenta. *Journal of Clinical Endocrinology and Medicine*, **73**, 882–887.

Hoffman, L.H. (1970) Placentation in the garter snake, *Thamnophis sirtalis*. *Journal of Morphology*, **131**, 57–88.

Hoffmann, L.H. and Wooding, F.B.P. (1993) Giant and binucleate trophoblastic cells of mammals. *Journal of Experimental Zoology*, **266**, 559–577.

Hoffman, L.H., Winfrey, V.P., Anderson, T.Z. and Olson, G.E. (1990a) Uterine receptivity to implantation in the rabbit: evidence for a 42 kDa glycoprotein as a marker of receptivity. *Trophoblast Research*, **4**, 243–258.

Hoffman, L.H., Winfrey, V.P. and Hoos, P.C. (1990b) Sites of endometrial vascular leakage during implantation in the rabbit. *Anatomical Record*, **227**, 47–61.

Hogarth, P.J. (1976) *Viviparity*, Studies in Biology No. 75. Arnold, London.

Hoshina, M., Boothby, M. and Boime, I. (1982) Cytological localization of chorionic gonadotrophin-α and placental lactogen mRNAs during development of the human placenta. *Journal of Cellular Biology*, **93**, 190–198.

Hradecky, P., Mossman, H.W. and Stott, G.G. (1988) Comparative histology of antelope placentomes. *Theriogenology*, **29**, 693–714.

Humblot, P., De Montigny, G., Jeanguyot, N., Tetodoie, F., Payen, B., Thibier, M. and Sasser, R.G. (1990) PSPB and progesterone concetrations in french alpine goats throughout gestation. *Journal of Reproduction and Fertility*, **89**, 205–212.

Hunt, J.S. and Hsi, B.L. (1990) Review. Evasive strategies of trophoblast cells: selective ex-

pression of membrane antigens. *American Journal of Reproductive Immunology*, **23**, 57–63.

Hunt, J.S., Manning, L.S. and Wood, G.W. (1984) Macrophages in murine uterus are immunosuppressive. *Cellular Immunology*, **85**, 499–510.

Hunt, J.S., Fishback, J.L., Chumbley, G. and Loke, Y.W. (1990) Identification of class I MHC mRNA in human first trimester trophoblast cells by in situ hybridisation. *Journal of Immunology*, **144**, 4420–4425.

Hunziker, R.D. and Wegman, T.G. (1986) Placental immunoregulation. *Clinical Reviews in Immunology*, **6**, 245–285.

Ilgren, E.B. (1983) Review Article: Control of trophoblast growth. *Placenta*, **4**, 307–328.

Imakawa, K., Anthony, R.V., Kazemi, M., Marotti, K.K., Polites, H.G. and Roberts, R.M. (1987) Interferon like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. *Nature*, **330**, 377–379.

Imakawa, K., Hansen, T.R., Malathy, P.V., Anthony, R.V., Polites, H.G., Marotti, K.R. and Roberts, R.M. (1989) Molecular cloning and characterisation of cDNAs corresponding to BTP-1; a comparison with OTP-1 and bovine interferon α_{II}. *Molecular Endocrinology*, **3**, 127–139.

Ing, N.H. and Roberts, R.M. (1989) The major progesterone modulated proteins secreted into the sheep uterus are members of the serpin superfamily of serine protease inhibitors. *Journal of Biological Chemistry*, **264**, 3372–3379.

Ingermann, R.L. (1987) Control of placental glucose transfer. *Placenta*, **8**, 557–571.

Johns, R. and Renegar, R.H. (1990) Ultrastructural morphology and relaxin immunolocalisation in giant trophoblast cells of the golden hamster placenta. *American Journal of Anatomy*, **189**, 167–178.

Johnson, S.A. and Wooding, F.B.P. (1988) Synthesis and storage of chorionic gonadotrophin and placental lactogen in human syncytiotrophoblast. *Journal of Physiology*, **403**, 29P.

Jollie, W.P. (1969) Nuclear and cytoplasmic fine structure in the trophectoderm giant cells of rat placenta. *Anatomical Record*, **65**, 1–14.

Jollie, W.P. (1979) The fine structure of placental junctional zone cells during prolonged pregnancy in rats. *Acta Anatomica*, **105**, 386–400.

Jollie, W.P. and Craig, S.S. (1979) The fine structure of the placental junctional zone cells during prolonged pregnancy in rats. *Acta Anatomica*, **105**, 386–400.

Jollie, W.P. and Jollie, L.G. (1967) Electron microscopic observations on the yolk sac of the spiny dogfish Squalus acanthias. *Journal of Ultrastructure Research*, **18** 102–126.

Jones, C.J.P. and Fox, H. (1991) Ultrastructure of the normal human placenta. *Electron microscopy Reviews*, **4**, 129–178.

Jones, R.E. and Guillette, L.J. (1982) Hormonal control of oviposition and parturition in lizards. *Herpetologica*, **38**, 80–93.

Kalter, S.S., Helmke, R.J., Panigel, M., Heberling, R.C., Felsburg, P.J. and Axelrod, L.R. (1973) Observation of apparent C type particles in Baboon (*Papio Cyno Cephalaus*) placentas. *Science (Washington)*, **179**, 1332–1333.

Kanbour, A., Ho, H.N., Misra, D.N., Macpherson, T.A., Kinz, H.W. and Gill, T.O. (1987) Differential expression of MHC class I antigens on the placenta of the rat. *Journal of Experimental Medicine*, **166**, 1882–1887.

Kappes, S.M., Warren, W.C., Pratt, S.L., Liang, R. and Anthony, R.V. (1992) Quantification and cell localisation of OPL mRNA expression during mid and late gestation. *Endocrinology*, **131**, 2829–2838.

Kasturirangan, L.R. (1952) The allantoplacenta of the sea snake Hydrophis cyanocinctus Daudin. *Journal of the Zoological Society of India*, **3**, 277–290.

Kaufmann, M.H. (1983) Origin, properties and fate of trophoblast in the mouse, in *Biology of Trophoblast* (eds C. Loke and A. Whyte), Elsevier, Amsterdam, 23–70.

Kaufmann, P. (1981a) Electron microscopy of the guinea pig placental membranes. *Placenta*, **2**, 3–10.

Kaufmann, P. (1981b) Entwicklung der Plazenta, in *Die Plazenta des Menschen*, (eds V. Becker, T.H. Schiebler and F. Kubli), Thieme, New York, pp. 13–50.

Kaufmann, P. (1982) Development and differentiation of the human placental villous tree. *Bibliotheca Anatomica*, **22**, 29–39.

Kaufmann, P. (1985) Basic morphology of the fetal and maternal circuits in the human placenta. *Contributions to Gynecology and Obstetrics*, **13**, 5–17.

Kaufmann, P., Bruns, V., Leiser, R., Luckhard, M. and Winterhager, E. (1985) The fetal vasculature of term human placental villi. II. Intermediate and terminal villi. *Anatomy and Embryology*

Kearns, M. and Lala, P.K. (1983) Life history of decidual cells: a review. *American Journal of*

Reproduction and Immunological Microbiology, **3**, 78–82.

Kellas, L.M. (1966) The placenta and foetal membranes of the antelope Ourebia ourebi (Zimmerman). *Acta Anatomica*, **64**, 390–445.

Kennedy, T.G. (1983) Embryonic signals and the initiation of blastocyst implantation. *Australian Journal of Biological Science*, **36**, 531–543.

Kennedy, T.G. and Lukash, L.A. (1982) Induction of decidualisation in rats by the intrauterine infusion of prostaglandins. *Biology of Reproduction*, **27**, 253–260.

Keys, J.L. and King, G.J. (1988) Morphological evidence for increased uterine vascular permeability at the time of embryonic attachment in the pig. *Biology of Reproduction*, **39**, 473–487.

Keys, J.L. and King, G.J. (1989) Structural changes in the luminal epithelium of the porcine uterus between days 10 and 19 of the estrous cycle. *American Journal of Anatomy*, **185**, 42–57.

Keys, J.L. and King, G.J. (1990) Microscopic examination of procine conceptus–maternal interface between days 10 and 19 of pregnancy. *American Journal of Anatomy*, **188**, 221–228.

Keys, J.L., King, C.J. and Kennedy, T.G. (1986) Increased uterine vascular permeability at the time of embryonic attachment in the pig. *Biology of Reproduction*, **34**, 405–411.

Keys, J.L., King, G.J. and Laforest, J.P. (1989) Autofluorescence of the porcine endometrium during early pregnancy. *Biology of Reproduction*, **40**, 220–222.

Kimber, S.J. and Lindenberg, S. (1990) Hormonal control of a carbohydrate epitope involved in implantation in mice. *Journal of Reproduction and Fertility*, **89**, 13–21.

Kimberley, D.J. and Thornburg, K.L. (1989) Electronmicroscopical tracers in the uterine epithelium of the pregnant guinea pig. *Placenta*, **10**, 531–543.

Kimura, K. and Uchida, T. (1984) Development of the main and accessory placentae in the Japanese long fingered bat *Miniopterus schreibersii fuliginosus*. *Journal of Reporoduction and Fertility*, **71**, 119–126.

Kimura, K. and Uchida, T.A. (1983) Electron microscopical observations of delayed implantation in the Japanese long fingered bat *Miniopterus schreibersii fuliginosus*. *Journal of Reproduction and Fertility*, **69**, 187–193.

King, A. and Loke, Y.W. (1993) Effect of IFN and α on killing of human trophoblast by decidual LAK cells. *Journal of Reproductive Immunology*, **23**, 51–62.

King, B.F. (1977) An electronmicroscopic study of absorption of peroxidaseconjugated immunoglobulin G by guinea pig visceral yolk sac *in vitro*. *American Journal of Anatomy*, **148**, 447–456.

King, B.F. (1984) The fine structure of the placenta and chorionic vesicles of the bush baby Galago crassicaudata. *American Journal of Anatomy*, **169**, 101–117.

King, B.F. (1986) Morphology of the placenta and fetal membranes, in *Comparative Primate Biology*, Vol. 3 (eds W.R. Dukelow and J.E. Erwin), Wiley, New York, *Reproduction and Development*, pp. 311–331.

King, B.F. (1992) Ultrastructural evidence for transtrophoblastic channels in the hemomonochorial placenta of the Degu (*Octodon degus*). *Placenta*, **13**, 35–41.

King, B.F. and Enders, A.C. (1971) Protein absorption by the guinea pig chorioallantoic placenta. *American Journal of Anatomy*, **130**, 409–430.

King, B.F. and Hastings, R.A. (1977) The comparative fine structure of the interhemal membrane of chorioallantoic placentas from six genera of myomorph rodents. *American Journal of Anatomy*, **149**, 165–180.

King, B.F. and Mossman, H.W. (1974) The fetal membranes and unusual giant cell placenta of the jerboa (*Jaculus*) and jumping mouse (*Zapus*). *American Journal of Anatomy*, **140**, 405–432.

King, B.F. and Tibbitts, F.D. (1969) The ultrastructure of the placental labyrinth in the kangaroo rat Dipodomys. *Anatomical Record*, **163**, 543–554.

King, B.F. and Tibbitts, F.D. (1976) The fine structure of the chinchilla placenta. *American Journal of Anatomy*, **145**, 33–56.

King, B.F., Enders, A.C. and Wimsatt, W.A. (1978) The annular hematoma of the shrew yolk sac placenta. *American Journal of Anatomy*, **152**, 45–58.

King, B.F., Pinheiro, P.B.N. and Hunter, R.L. (1982) The form of the placental labyrinth in the sloth Bradypus tridactylis. *Anatomical Record*, **202**, 15–22.

King, G.J. and Ackerley, C.A. (1985) Demonstration of oestrogens on developing pig trophectoderm and yolk sac endoderm between days 10 and 16. *Journal of Reproduction and Fertility*, **73**, 361–367.

King, G.J. and Atkinson, B.A. (1987) The bovine intercaruncular placenta throughout gestation. *Animal Reproduction Science*, **12**, 241–254.

King, G.J., Atkinson, B.A. and Robertson, H.A. (1979) Development of the bovine placentome during the second month of gestation. *Journal of Reproduction and Fertility*, **55**, 173–180.

King, G.J., Atkinson, B.A. and Robertson, H.A. (1981) Development of the intercaruncular areas during early gestation and the establishment of the bovine placenta. *Journal of Reproduction and Fertility*, **61**, 469–474.

Kirby, D.R.S. (1963) The development of mouse blastocysts transplanted to the scrotal and cryptorchid testis. *Journal of Anatomy*, **97**, 119–130.

Kirby, D.R.S. (ed.) (1965) The 'invasiveness' of the trophoblast, in *The early Conceptus, normal and abnormal* (ed. W. Wallace Park), Livingstone, London, pp. 68–73.

Kisalus, L.L., Hew, J.C. and Little, C.D. (1987) Immunolocalisation of extracellular matrix proteins and collagen synthesis in first trimester human decidua. *Anatomical Record*, **218**, 402–415.

Kishi, K., Ogren, L., Southard, J.N. and Talamantes, F. (1988) Pituitary factors regulating mouse placental lactogen II secretion during the last half of pregnancy in mice. *Endocrinology*, **122**, 2309–2317.

Kiso, Y., Yasufuku, K., Matsuda, H. and Yamavchi, S. (1990) Existence of an endothelio-endothelial placenta in the insectivore Suncus murinus. *Cell and Tissue Research*, **262**, 195–197.

Kitchen, H. and Bunn, H.F. (1975) Ontogeny of equine haemoglobins. *Journal of Reproduction and Fertility*, Suppl. 23, 595–598.

Kliman, H.J., Nestler, J.E., Sermasi, E., Sanger, J.M. and Strauss, J.F. (1986) Purification, characterisation and *in vitro* differentiation of cytotrophoblast from human term placenta. *Endocrinology*, **118**, 1567–1582.

Klopper, A. and Ahmed, A.G. (1985) New applications for the assay of placental proteins. *Placenta*, **6**, 173–184.

Knickerbocker, J.J., Thatcher, W.W., Berger, F.W., Drost, M., Barron, D.H., Fincher, K.B. and Roberts, R.M. (1986) Proteins secreted by day 16 to 18 bovine conceptuses extend CL function in cows. *Journal of Reproduction and Fertility*, **77**, 381–391.

Knight, P.M., Lombardi, J., Wourms, J.P. and Burns, J.R. (1985) Follicular placenta and embryonic growth of the viviparous four eyed fish (*Anableps*). *Journal of Morphology*, **185**, 131–142.

Kramer, B., Stein, B.A. and Vanderwalt, L.A. (1990) Exogenous gonadotropins serum oestrogen and progesterone and the effect on endometrial morphology in the rat. *Journal of Anatomy*, **173**, 177–186.

Krcek, J. and Clark, D.A. (1985) Selective localisation of a bone marrow cell subpopulation at the implantation site in murine decidua. *American Journal of Reproduction and Immunological Microbiology*, **7**, 95–98.

Kunz, Y.W. (1971) Histological study of the greatly enlarged pericardial sac in the embyro of the viviparous telost Lebistes reticulatus. *Revue Suisse Zoologie*, **78**, 187–207.

Kurman, R.J., Main, C.S. and Chen, H.C. (1984) Intermediate trophoblast: a distinctive form of trophoblast with specific morphological biochemical and functional features. *Placenta*, **5**, 349–370.

Lala, P.K. (1989) Similarities between immunoregulation in pregnancy and malignancy: the role of prostaglandin E2. *American Journal of Reproductive Immunology*, **20**, 147–152.

Lala, P.K., Chatterjee, Hasrouni.S., Kearns, M., Montgomery, B. and Colavicenzo, V. (1983) Immunobiology of the fetomaternal interface. *Immunological Reviews*, **75**, 87–116.

Larkin, L.H. and Flickinger, C.J. (1969) Ultrastructure of the metrial gland cell in the pregnant rat. *American Journal of Anatomy*, **126**, 337–354.

Larsen, J.F. (1961) Electron microscopy of the implantation site in the rabbit. *American Journal of Anatomy*, **109**, 319–334.

Lawn, A.M., Chiquoine, A.D. and Amoroso, E.C. (1969) The development of the placenta in the sheep and goat: an electron microscopic study. *Journal of Anatomy*, **105**, 557–578.

Leach, L., Eaton, B.M., Firth, J.A. and Contractor, S.F. (1990) Uptake and intracellular routing of peroxidase conjugated IgG by the perfused human placenta. *Cell and Tissue Research*, **261**, 383–388.

Ledger, W.L., Ellwood, D.A. and Taylor, M.J. (1983) Cervical softening in late pregnant sheep by infusion of PGE_2into a cervical artery. *Journal of Reproduction and Fertility*, **69**, 511–523.

Lee, C.S., Gogolin-Ewens, K., White, T.R. and Brandon, M.R. (1985) Studies on the distribution of binucleate cells in the placenta of sheep with a monoclonal antibody SBU–3. *Journal of Anatomy*, **140**, 565–576.

Lee, C.S., Gogolin-Ewens, K. and Brandon, M. (1986a) Comparative studies on the distribution of binucleate cells in the placentae of the deer

and cow using the monoclonal antibody SBU3. *Journal of Anatomy*, **147**, 163–180.

Lee, C.S., Wooding, F.B.P. and Brandon, M.R. (1986b) Light and electron microscope immunocytochemical studies on the role of binucleate cells in villus growth in goat placentomes. *Journal of Submicroscopical Cytology*, **18**, 661–672.

Lee, C.S., Wooding, F.B.P. and Brandon, M.R. (1986c) Immunogold co-localization of ovine placental lactogen and the antigen recognized by the SBU-3 monoclonal antibody in sheep placental granules. *Journal of Reproduction and Fertility*, **78**, 653–662.

Lee, C.S., Wooding, F.B.P. and Brandon, M.R. (1986d) Ultrastructural immunogold investigations of the function and diversity of binucleate cells in the sheep placenta using a monoclonal antibody. *Placenta*, **7**, 495–504.

Lee, C.S., Ralph, M.M., Gogolin Ewens, K.J. and Brandon, M.R. (1990) Monoclonal antibody identification of cells dissociated from the sheep placentomal trophoblast. *Journal of Histochemistry and Cytochemistry*, **38**, 649–652.

Lee, C.S., Meeusen, E., Gogolin Ewens, K. and Brandon, M.R. (1992) Quantitative and qualitative changes in the intraepithelial lymphocyte population in the uterus of nonpregnant and pregnant sheep. *American Journal of Reproductive Immunology*, **28**, 90–96.

Lee, S.Y., Mossman, H.W., Mossman, A.S. and DelPino, G. (1977) Evidence for a specific implantation site in ruminants. *American Journal of Anatomy*, **150**, 631–640.

Lee, S.Y., Anderson, J.W., Scott, G.L. and Mossman, H.W. (1983) Ultrastructure of the placenta and fetal membranes of the dog. II. The yolk sac. *American Journal of Anatomy*, **166**, 313–327.

Legrand, C. (1974) Origine et évolution des cellules trophoblastiques intra arterielles, chez le rat: étude autoradiographique. *Journal of Experimental Embryology and Medicine*, **31**, 693–705.

Leiser, R. (1975) Kontaktaufnahme zwischen trophoblast und uterus epithel wahrend der frühen implantation beim rind. *Anatomy Histology Embryology*, **4**, 63–86.

Leiser, R. (1979) Blastocysten implantation bei der Hauskatze. Licht und Elektronen optische untersuchungen. *Anatomy Histology Embryology*, **8**, 79–96.

Leiser, R. (1987) Mikrovaskularisation der Ziegenplazenta, dargestellt mit Rasterelektronisch untersuchten Gefassausgussen. *Schweizwer Archiv fur Tierheilkunde*, **129**, 59–74.

Leiser, R. and Dantzer, V. (1988) Structural and functional aspects of porcine placental vasculature. *Anatomy and Embryology*, **177**, 409–419.

Leiser, R. and Enders, A.C. (1980a) Light and electron microscopy study of the near term paraplacenta of the domestic cat. 1. Polar zone and paraplacental functional areas. *Acta Anatomica*, **106**, 293–311.

Leiser, R. and Enders, A.C. (1980b) Light and electron microscopy study of the near term paraaplacenta of the domestic cat. 2. paraplacental hematoma. *Acta Anatomica*, **106**, 312–326.

Leiser, R. and Kohler, T. (1983) The blood vessels of the cat girdle placenta. Observations on corrosion casts. SEM and histological studies. I. Maternal vasculature. *Anatomy and Embryology*, **167**, 85–93.

Leiser, R. and Kohler, T. (1984) The blood vessels of the cat girdle placenta. observations on corrosion casts, scanning electron microscopical and histological studies. II. Fetal vasculature. *Anatomy and Embryology*, **170**, 209–216.

Lemons, J.A., Adcock III, E.W., Jones Jr., M.D., Naughton, M.A., Meschia, G. and Battaglia, F.C. (1976) Umbilical uptake of amino acids in the unstressed fetal lamb. *Journal of Clinical Investigation*, **58**, 1428–1434.

Lennep, E.W. (1961) The histology of the placenta of the one humped camel (*Camelus dromedarius*) during the first half of pregnancy. *Acta Morphological Neerlandica Scandinavica*, **4**, 180–193.

Lifsey, B.J., Baumbach, G.A. and Godkin, J.D. (1989) Isolation characterisation and immunocytochemical localisation of bovine trophoblast protein-1. *Biology of Reproduction*, **40**, 343–352.

Liggins, G.C. (1981) Endocrinology of parturition, in *Fetal Endocrinology* (eds M.J. Novy and J.A. Resko), Academic Press, New York, pp. 211–237.

Lin, T.M., Halbert, S.P., Kiefer, D., Spellacy, W.N. and Gall, S. (1974) Characterization of four human pregnancy-associated plasma proteins. *American Journal of Obstetrics and Gynecology*, **118**, 223–236.

Lindenberg, S., Hyttel, P., Sjogren, A. and Greve, T. (1989) A comparative study of attachment of human bovine and mouse blastocysts to uterine epithelial monolayers. *Human Reproduction*, **4**, 446–456.

Loke, Y.W. and King, A. (1989) Immunology of

pregnancy: quo vadis? *Human Reproduction*, **4**, 613–615.

Loke, Y.W., Burland, K. and Butterworth, B. (1986) Antigen bearing trophoblast in human placental implantation site, in *Reproduction and Immunology* (eds D.A. Clark and B.A. Croy), Elsevier, London, pp. 53–59.

Lombardi, J. and Wourms, J.P. (1985a) The trophotaenial placenta of a viviparous Goodeid Fish. I. Ultrastructure of the internal ovarian epithelium: the maternal component. *Journal of Morphology*, **184**, 277–292.

Lombardi, J. and Wourms, J.P. (1985b) The trophotaenial placenta of a viviparous Goodeid Fish. II. Ultrastructure of trophotaeniae: the embryonic component. *Journal of Morphology*, **184**, 293–309.

Lombardi, J. and Wourms, J.P. (1985c) The trophotaenial placenta of a viviparous Goodeid Fish. III. Protein uptake by trophotaeniae: the embryonic component. *Journal of Experimental Zoology*, **236**, 165–179.

Lowe, K.C., Beck, N.F.G., McNaughton, D.C., Jansen, C.A.M., Thomas, A.L., Nathanielz, P.W., Mallon, K. and Steven, D.H. (1979) Ultrastructural changes in the placenta of the ewe after long term intravascular infusion of 2-bromo-β-ergocryptine (CB154) into mother or fetus. *Quarterly Journal of Experimental Physiology*, **64**, 253–262.

Luckett, W.P. (1976) Cladistic relationship among primate higher categories: evidence from fetal membranes and placenta. *Folia Primatologia*, **25**, 245–276.

Luckett, W.P. (1977) Ontogeny of amniote fetal membranes and their application to phylogeny, in *Major Patterns in Vertebrate Evolution* (eds M.K. Hecht, P.C. Goody and B.M. Hecht), Plenum Press, New York, pp.439–516.

Luckett, W.P. (1978) Origin and differentiation of the yolk sac and embryonic mesoderm in presomite human and Rhesus monkey embryos. *American Journal of Anatomy*, **152**, 59–98.

Luckhardt, M., Kaufmann, P. and Elger, W. (1985) The structure of the Tupaia placenta. I. Histology and vascularisation. *Anatomy and Embryology*, **171**, 201–210.

Ludwig, K.S. (1962) Zur feinstruktur der maternofetalen verbindung in placentom des schafes (*Ovis aries*. L.). *Experientia*, **18**, 212–213.

Ludwig, K.S. (1968) Zur vergleichenden Histologie des Allantochorion. *Revue Suisse de Zoologie*, **75**, 819–831.

MacDonald, A.A. (1975) Uterine vasculature of the pregnant pig: a scanning electron microscopy study. *Anatomical Record*, **184**, 689–698.

MacDonald, A.A. (1981) The vascular anatomy of the pig placenta: a scanning electron microscope study. *Acta Morphologica Neerlandica Scandinavica*, **19**, 171–172.

McGovern, P.T. (1973) The effect of maternal immunity on the survival of goat x sheep hybrid ♂ embryos. *Journal of Reproduction and Fertility*, **34**, 215–220.

Magness, R.R., Mitchell, M.D. and Rosenfeld, C.R. (1990) Uteroplacental production of eicosanoids in ovine pregnancy. *Prostaglandins*, **39**, 75–88.

Makowski, E.L. (1968) Maternal and fetal vascular nets in placentas of sheep and goats. *American Journal of Obstetrics and Gynecology*, **100**, 283–288.

Malassine, A. (1974) Evolution ultrastructurale du labyrinthe de placenta de chatte. *Anatomy and Embryology*, **146**, 1–20.

Malassine, A. and Leiser, R. (1984) Morphogenesis and fine structure of the near term placenta of Talpa europea. 1. Endotheliochorial labyrinth. *Placenta*, **5**, 145–158.

Martal, J. and Djiane, J. (1977) The production of chorionic somatomammotrophin in sheep. *Journal of Reproduction and Fertility*, **49**, 285–289.

Martal, J. and Lacroix, M.C. (1978) Production of chorionic somatomammotrophin (OCS): fetal growth of the placenta and corpus luteum in ewes treated with 2-bromo-β-ergocryptine (BC). *Endocrinology*, **103**, 193–199.

Martal, J., Djiane, J. and Dubois, M.P. (1977) Immunofluorescent localisation of ovine placental lactogen. *Cell and Tissue Research*, **184**, 427–433.

Martal, J., Lacroix, M.C., Loudes, C., Saunier, M. and Wintenberger-Torres, S. (1979) Trophoblastin, an antiluteolytic protein present in early pregnancy in sheep. *Journal of Reproduction and Fertility*, **56**, 63–73.

Martin, C.B. (1981) Models of placental blood flow. *Placenta*, Suppl. 1, 65–80.

Martinek, J.J. (1970) Fibrinoid and the fetal–maternal interface of the rat placenta. *Anatomical Record*, **166**, 587–604.

Maslar, I.A., Powers, J., Craddock, P. and Ansbacher, K. (1986) Decidual prolactin production by organ cultures of human endometrium: effects of intermittent progesterone treatment. *Biology of Reproduction*, **34**, 741–750.

Mathialagan, N., Bixby, J.A. and Roberts, R.M.

(1992) Expression of IL6 in porcine, ovine and bovine preimplantation conceptuses. *Molecular Reproduction and Development*, **32**, 324–330.

Matre, R., Tonder, O. and Endresen, C. (1975) Fe receptors in human placenta. *Scandinavian Journal of Immunology*, **4**, 741–745.

Mattson, B.A., Overstrom, E.W. and Albertini, D.E. (1990) Transitions in trophectodermal cellular shape and cytoskeletal organisation in the elongating pig blastocyst. *Biology of Reproduction*, **42**, 195–205.

Mayhew, T.M., Joy, C.F. and Haas, J.D. (1984) Structure–function correlation in the human placenta: the morphometric diffusing capacity for oxygen at full term. *Journal of Anatomy*, **139**, 691–708.

Mayhew, T.M., Jackson, M.R. and Haas, J.D. (1986) Microscopical morphology of the human placenta and its effects on oxygen diffusion: a morphometric model. *Placenta*, **7**, 121–131.

Mazariegos, M.R., Leblond, C.P. and van der Rest, M. (1987) Radioautographic tracing of ^{3}H proline in the endodermal cells of the parietal yolk sac as an indicator of the biogenesis of basement membrane components. *American Journal of Anatomy*, **179**, 79–93.

Mead, R.A. (1993) Embryonic diapause in vertebrates. *Journal of Experimental Zoology*, **266**, 629–641.

Medawar, P.B. (1953) Some immunological and endocrinology problems raised by the evolution of viviparity in vertebrates. *Symposia Society for Experimental Biology*, **7**, 328–338.

Meinecke-Tillmann, S. (1984) Experimental chimaeras – removal of reproductive barrier between sheep and goat. *Nature*, **307**, 637–638.

Meites, J., Webster, H.D., Young, F.W., Thorp, F. and Hatch, R.N. (1951) Effects of corpora lutea removal and replacement with progesterone on pregnancy in goats. *Journal of Animal Science*, **10**, 411–416.

Merker, H.J., Bremer, D., Barach, H.J. and Gossrau, R. (1987) The basement membrane of the persisting maternal blood vessels in the placenta of Callithrix jacchus. *Anatomy and Embryology*, **176**, 87–97.

Metcalfe, J., Moll, W., Bartels, H., Hilpert, P. and Parer, J.T. (1965) Transfer of carbon monoxide and nitrous oxide in the artifically perfused sheep placenta. *Circulation Research*, **16**, 95–101.

Metz, J. (1980) On the development of the rat placenta I. Differentiation and functional alterations of labyrinthine layers II and III. *Anatomy and Embryology*, **159**, 289–305.

Metz, J., Heinrich, D. and Forssmann, W.G. (1976) Gap junctions in haemodi-, and trichorial placentas. *Cell and Tissue Research*, **171**, 305–316.

Metz, J., Aoki, A. and Forssman, W.G. (1978) Studies on the ultrastructure and permeability of the haemotrichorial placenta. I. Intercellular junctions of layer I and tracer administration into the maternal compartment. *Cell and Tissue Research*, **192**, 391–407.

Meuris, S., Soumenkoff, G., Malengrean, A. and Robyn, C. (1980) Immunoenzymological localisation of prolactin like immunoreactivity in decidual cells of the endometrium from pregnant and non pregnant women. *Journal of Histochemistry and Cytochemistry*, **28**, 1347–1350.

Michael, C.A. and Schofield, B.M. (1968) The influence of ovarian hormones on the actomyosin content and the development of tension in uterine muscle. *Journal of Endocrinology*, **44**, 501–511.

Michael, K., Ward, B.S. and Moore, W.M.O. (1985) *In vitro* permeability of the pig placenta in the last third of gestation. *Biology of the Neonate*, **47**, 170–178.

Miller, W.L. and Eberhardt, N.L. (1983) Structure and evolution of the growth hormone gene family. *Endocrine Reviews*, **4**, 97–130.

Milosavljevic, M., Duello, T.M. and Schuler, L.A. (1989) In situ localisation of the prolactin related mRNAs to binuleate cells of bovine placentomes. *Endocrinology*, **125**, 883–889.

Mitchell, B.S., Craggs, R.I. and Peel, S. (1981) Localisation of immunoglobulins (IgG) in granulated metrial gland cells of deciduomata of the pseudopregnant rat. *Journal of Reproductive Immunology*, **3**, 237–241.

Mitchell, M.D. and Flint, A.P.F. (1978a) Prostaglandin production by intra-uterine tissues from periparturient sheep: use of a superfusion technique. *Journal of Endocrinology*, **76**, 111–121.

Mitchell, M.D. and Flint, A.P.F. (1978b) Use of meclofenamic acid to investigate the role of prostaglandin biosynthesis during induced parturition in sheep. *Journal of Endocrinology*, **76**, 101–109.

Molina, R.D., Meschia, G., Battaglia, F.C. and Hay, W.W. (1991) Gestational maturation of placental glucose transfer capacity in sheep. *American Journal of Physiology*, **261**, 697–704.

Moll, W. (1972) Gas exchange in concurrent, countercurrent and crosscurrent flow systems. The

concept of the fetoplacental flow unit, in *Respiratory Gas Exchange and Blood Flow in the Placenta*. (eds L.D. Longo and H. Bartels), US DHEW Publication No. (NIH), pp. 73–361.

Moll, W. (1985) Physiological aspects of placental ontogeny and phytogeny. *Placenta*, **6**, 141–154.

Moore, H.D.M., Gems, S. and Hearn, J.P. (1985) Early implantation stages in the marmoset monkey (*Callithrix jacchus*). *American Journal of Anatomy*, **172**, 265–278.

Morgan, G. and Wooding, F.B.P. (1983) Cell migration in the ruminant placenta, a freeze fracture study. *Journal of Ultrastructure Research*, **83**, 148–160.

Morgan, G., Wooding, F.B.P. and Brandon, M.R. (1987) Immunogold localisation of placental lactogen and the SBU3 antigen by ultracryomicrotomy at implantation in the sheep. *Journal of Cell Science*, **88**, 503–512.

Morgan, G., Whyte, A. and Wooding, F.B.P. (1990) Characterisation of the synthetic capacities of isolated binucleate cells from sheep and goats. *Anatomical Record*, **226**, 27–36.

Morrish, D.W., Marusyk, H. and Siy, O. (1987) Demonstration of specific secretory granules for human choriogonadotrophin in the placenta. *Journal of Histochemistry and Cytochemistry*, **35**, 93–101.

Morrish, D.W., Marusyk, H. and Bhardwaj, D. (1988) Ultrastructural localisation of hPL in distinctive granules in human term placenta: comparison with granules containing hCG. *Journal of Histochemistry and Cytochemistry*, **36**, 193–197.

Morriss, F.H. and Boyd, R.D.H. (1988) Placental transport, in *Physiology of Reproduction*, Chapter 50 (eds E. Knobil and J. Neill), Raven Press, New York.

Morton, W.R.M. (1961) Observations on the full term foetal membranes of the three members of the Camelidae (*Camelus dromedarius* L., C. *bactrianus* L. and *Lama glama* L.). *Journal of Anatomy*, **95**, 200–209.

Mossman, H.W. (1926) The rabbit placenta and the problem of placental transmission. *American Journal of Anatomy*, **37**, 433–497.

Mossman, H.W. (1937) Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Carnegie Institute Contributions to Embryology*, **26**, 129–246.

Mossman, H.W. (1965) The principal interchange vessels of the chorioallantoic placenta of mammals, in *Organogenesis* (eds R.L. De Haan and H. Ursprung), Holt Rinehart Wilson, New York, pp. 771–786.

Mossman, H.W. (1987) *Vertebrate Fetal Membranes*, Rutgers University Press, New Brunswick, New Jersey.

Moxon, L.A., Wildt, A.E. and Slade, B.S. (1976) Localization of proteins in coated micropinocytotic vesicles during transport across rabbit yolk sac endoderm. *Cell and Tissue Research*, **171**, 175–193.

Munro, H.N. (1980) Placenta in relation to nutrition. *Federation Proceedings*, **39**, 236–238.

Munro, H.N. (1986) Role of the placenta in ensuring fetal nutrition. *Federation Proceedings*, **45**, 2500–2501.

Munson, L., Wilkinson, J.E. and Schlafer, D.H. (1990) Effects of substrata on the polarisation of bovine endometrial epithelial cells *in vitro*. *Cell and Tissue Research*, **261**, 155–161.

Murthy, A.S., Schellenberg, C. and Friesen, H.G. (1982) Purification and characterisation of bovine placental lactogen. *Endocrinology*, **III**, 2117–2174.

Musah, A.I., Schwabe, C., Willham, R.L. and Anderson, L.L. (1987) Induction of parturition, progesterone secretion and delivery of placenta in beef heifers given relaxin with cloprostenol or dexamethasone. *Biology of Reproduction*, **37**, 797–803.

Nathanielsz, P.W. (1980) *Animal Models in Fetal Medicine*, Elsevier, North Holland, Amsterdam.

Nelson, D.M., Otman Nabi, J. and Curran, E.M. (1986) Morphology and glycoprotein synthesis of uterine gland epithelium in human basal plate at term: an ultrastructural and autoradiographic study. *Anatomical Record*, **216**, 146–153.

Nephew, K.P., McClure, K.E., Day, M.L., Xie, S., Roberts, R.M. and Pope, W.F. (1990) Effects of intramuscular administration of recombinant bovine interferon alpha-1 during the period of maternal recognition of pregnancy. *Journal of Animal Science*, **68**, 2776–2780.

Nieder, G.L. and Jenne, L. (1990) Production of mouse placental lactogen-1 by trophoblast giant cells in utero and *in vitro*. *Endocrinology*, **126**, 2809–2814.

O'Shea, J.D., Kleinfeld, F.G. and Morrow, H.A. (1983) Ultrastructure of decidualisation in the pseudopregnant rat. *American Journal of Anatomy*, **166**, 271–298.

Ockleford, C.D. and Menon, G. (1977) Differentiated regions of human placental cell surface associated with exchange of materials between

maternal and fetal blood: a new organelle and the binding of iron. *Journal of Cell Science*, **25**, 279–291.

Oduor-Okelo, D. (1984a) Histology of chorioallantoic placenta of the golden rumped elephant shrew (*Rhyncocyon chrysopygus*). *Anatomische Anzeiger*, **157**, 395–407.

Oduor-Okelo, D. (1984b) An electron microscopy study of the chorioallantoic placenta and subplacenta of the cane rat (*Thryonomys swinderianus Temminck*). *Placenta*, **5**, 433–442.

Oduor-Okelo, D. and Neaves, W.B. (1982) The chorioallantoic placenta of the spotted hyena (*Crocuta, crocuta Erxleben*): an electron microscopy study. *Anatomical Record*, **204**, 215–222.

Oduor-Okelo, D., Musewe, U.O. and Gombe, S. (1983) Electron microscopic study of the chorioallantoic placenta of the rock hyrax (*Heterohyrax brunei*). *Journal of Reproduction and Fertility*, **68**, 311–316.

Ogren, L. and Talamantes, F. (1988) Placental lactogens of pregnancy and their cellular source. *International Review of Cytology*, **112**, 1–65.

Ogren, L., Southard, J.N., Colosi, P., Linzer, D.I.H. and Talamantes, F. (1989) Mouse placental lactogen-I: RIA and gestational profiles in maternal serum. *Endocrinology*, **125**, 2253–2257.

Ogura, A., Nishida, T., Hayashi, Y. and Mochida, K. (1991) The development of the uteroplacental vascular system in the golden hamster *Mesocricetus auratus*. *Journal of Anatomy*, **175**, 65–77.

Oksenberg, J.R., Moryosef, S., Persitz, E., Schenker, Y., Mozes, E. and Brantbar, C. (1986) Antigen presenting cells in human decidual tissue. *American Journal of Reproduction and Immunological Microbiology*, **11**, 82–88.

Owens, J.A. (1991) Endocrine and substance control of fetal growth: placental and maternal influences and IGFs. *Reproduction, Fertility and Development*, **3**, 501–518.

Owers, N.O. (1960) The endothelio-endothelial placenta of the Indian musk shrew, Suncus murinus – a new interpretation. *American Journal of Anatomy*, **106**, 1–25.

Owiti, G.E.O., Tarava, R.P. and Hendrickx, A.G. (1989) Fetal membranes and placenta of the african green monkey *Cercopithecus daethiops*. *Anatomy and Embryology*, **179**, 591–604.

Owiti, G.E.O., Cukierski, M., Tarara, R.P., Enders, A.C. and Hendrick, A.G. (1986) Early placentation in the African green monkey (*Cercopithecus aethiops*). *Acta Anatomica*, **127**, 184–194.

Padykula, H.A. and Taylor, J.M. (1976) Ultrastructural evidence for loss of the trophoblast layer in the chorioallantoic placenta of Australian bandicoots (*Marsupialia; Peramelidae*). *Anatomical Record*, **186**, 357–386.

Panigel, M. (1956) Contribution a l'étude de l'ovoviviparité chez les reptiles: Gestation et parturition chez le lézard *Zootoca vivipara*. *Annales des Sciences Naturelles Zoologie*, **XI 18**, 569–668.

Parr, E.L., Parr, M.B. and Young, J.D. (1987) Localisation of a pore forming protein (Perforin) in granulated metrial gland cells. *Biology of Reproduction*, **37**, 1327–1335.

Parr, M.B. and Parr, E.L. (1986) Permeability of the primary decidual zone (PDZ) in the rat uterus: studies using fluorescent labelled proteins and dextrans. *Biology of Reproduction*, **34**, 393–403.

Parr, M.B. and Parr, E.L. (1989) The implantation reaction, in *Biology of Uterus*, 2nd edn (eds R.M. Wynn and W.P. Jollie), Plenum Press, New York, pp. 233–278.

Parr, M.B., Tung, H.N. and Parr, E.L. (1986) The ultrastructure of the rat primary decidual zone. *American Journal of Anatomy*, **176**, 423–436.

Pashen, R.L., Sheldrick, E.L., Allen, W.R. and Flint, A.P.F. (1983) Dehydroepiandrosterone synthesis by the foetal foal and its importance as an oestrogen precursor. *Journal of Reproduction and Fertility*, Suppl. 32, 389–397.

Patillo, R.A., Hussa, R.O., Yorde, D.E. and Cole, L.A. (1983) Hormone synthesis by normal and neoplastic human trophoblast, in *Biology of Trophoblast* (eds Y.W. Loke and A. Whyte), Elsevier, Amsterdam, pp. 283–316.

Peel, S. (1989) Granulated metrial gland cells. *Advances in Anatomy, Embryology and Cell Biology*, **115**, 1–112.

Peel, S. and Stewart, I. (1979) Ultrastructural changes in the rat metrial gland in the latter half of pregnancy. *Anatomy and Embryology*, **155**, 209–219.

Peel, S. and Stewart, J. (1986) Oestrogen and the differentiation of granulated metrial gland cells in chimeric mice. *Journal of Anatomy*, **144**, 181–187.

Peel, S., Stewart, I.G. and Bulmer, D. (1979) Metrial gland cells in deciduomata of pseudopregnancy. *Journal of Anatomy*, **129**, 21–30.

Peel, S., Stewart, I.G. and Bulmer, D. (1983) Experimental evidence for the bone marrow origin of granulated metrial gland cells of the mouse uterus. *Cell and Tissue Research*, **233**, 647–656.

Perry, J.S. (1974) Implantation of fetal membranes and early placentation of the African elephant Loxodonta africana. *Philosophical Transactions of the Royal Society*, **B 269**, 109–135.

Perry, J.S. (1981) The mammalian fetal membranes. *Journal of Reproduction and Fertility*, **62**, 321–335.

Perry, J.S. and Rowlands, I.W. (1962) Early pregnancy in the pig. *Journal of Reproduction and Fertility*, **4**, 175–188.

Perry, J.S., Heap, R.B. and Ackland, N. (1975) The ultrastructure of the sheep placenta around the time of parturition. *Journal of Anatomy*, **120**, 561–570.

Pijnenborg, R., Dixon, G., Robertson, W.B. and Brosens, I. (1980) Trophoblast invasion of human decidua from 8–18 weeks of pregnancy. *Placenta*, **1**, 3–19.

Pijnenborg, R., Robertson, W.B., Brosens, I. and Dixon, G. (1981) Trophoblast invasion and the establishment of hemochorial placentation in man and laboratory animals. *Placenta*, **2**, 71–92.

Pijnenborg, R., Robertson, W.B., Brosens, I. and Dixon, G. (1983) Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. *Placenta*, **4**, 397–414.

Polge, C., Rowson, L.E.A. and Chang, M.C. (1966) The effect of reducing the number of embryos during the early stages of gestation on the maintenance of pregnancy in the pig. *Journal of Reproduction and Fertility*, **12**, 395–397.

Porter, D.G. (1967) Observations on the development of mouse blastocysts transferred to the testis and kidney. *American Journal of Anatomy*, **121**, 73–85.

Porter, D.G., Heap, R.B. and Flint, A.P.F. (1982) Endocrinology of the placenta and the evolution of viviparity. *Journal of Reproduction and Fertility*, Suppl. 31, 113–138.

Potts, D.M. (1968) The ultrastructure of implantation in the mouse. *Journal of Anatomy*, **103**, 77–90.

Power, G.G. and Gilbert, R.C. (1977) Umbilical vascular compliance in sheep. *American Journal of Physiology*, **233**, H660–H664.

Power, G.G. and Longo, L.D. (1973) Sluice flow in placenta: maternal vascular pressure effects on fetal circulation. *American Journal of Physiology*, **225**, 1490–1496.

Prasad, M.R.N., Mossman, H.W. and Scott, G.L. (1979) Morphogenesis of the fetal membranes of an American mole *Scalopus aquaticus*. *American Journal of Anatomy*, **155**, 31–68.

Pusey, J., Kelly, W.A., Bradshaw, J.M.C. and Porter, D.G. (1980) Myometrial activity and the distribution of blastocysts in the uterus of the rat: interference by relaxin. *Biology of Reproduction*, **23**, 394–397.

Ralph, M.M., Lee, C.S. and Thorburn, G.D. (1989) Identification and characterisation of monolayer cultures of sheep trophoblast cells maintained in bicameral culture chambers. *Biology of Reproduction*, **41**, 481–489.

Ramsey, E.M. (1967) Vascular Anatomy, in *Biology of the Uterus* (ed. R.M. Wynn), Plenum Press, New York, pp. 59–76.

Ramsey, E.M. (1982) *The Placenta, Human and Animal*, Prager Pub (CBS), New York.

Ramsey, E.M. and Donner, M.W. (1980) Placental vasculature and circulation, an atlas and textbook. *Thieme*, Stuttgart.

Ramsey, E.M., Houston, M.L. and Harris, J.W.S. (1976) Interactions of the trophoblast and maternal tissues in three closely related primate species. *American Journal of Obstetrics and Gynecology*, **124**, 647–652.

Rankin, J.H.G. (1978) Role of prostaglandins in the maintenance of the placental circulation. *Advances in Prostaglandin and Thromboxane Research*, **4**, 261–269.

Rankin, J.H.G. and McLaughlin, M.K. (1979) The regulation of the placental blood flows. *Journal of Developmental Physiology*, **1**, 3–30.

Rankin, J.H.G. and Phernetton, T.M. (1976) Effect of prostaglandin E_2 on ovine maternal placental blood flow. *American Journal of Physiology*, **231**, 754–765.

Rankin, J.H.G. and Schneider, J.M. (1975) Effect of surgical stress on the distribution of placental blood flows. *Respiration Physiology*, **24**, 373–383.

Rankin, J.H.G., Meschia, G., Makowski, E.L. and Battaglia, F.C. (1970) Macroscopic distribution of blood flow in the sheep placenta. *American Journal of Physiology*, **219**, 9–16.

Rasweiler, J.J. (1979) Early embryonic development and implantation in bats. *Journal of Reproduction and Fertility*, **56**, 403–416.

Rasweiler, J.J. (1990) Implantation, development of the fetal membranes and placentation in the captive black mastiff bat, *Molussus ater*. *American Journal of Anatomy*, **187**, 109–136.

Rasweiler, J.J. (1991) Development of the discoidal hemochorial placenta in the black mastiff bat Molossus ater: evidence for a role of maternal endothelial cells in the control of trophoblastic

growth. *American Journal of Anatomy*, **191**, 185–207.

Rasweiler, J.J. (1993) Pregnancy in chiroptera. *Journal of Experimental Zoology*, **266**, 495–513.

Rattray, P.V., Garrett, W.N., East, N.E. and Hinman, N. (1974) Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. *Journal of Animal Science*, **38**, 613–626.

Raub, T.J., Bazer, F.W. and Roberts, R.M. (1985) Localisation of the iron transport protein uteroferrin in the porcine endometrium and placenta using immunocolloid gold. *Anatomy and Embryology*, **171**, 253–268.

Redman, C.W.G., McMichael, A.J., Stirrat, G.M., Sunderland, C.A. and Ting, A. (1984) Class I MHC antigens on human extravillous trophoblast. *Immunology*, **52**, 457–468.

Reimers, T.J., Ullman, M.B. and Hansel, W. (1985) Progesterone and prostanoid production by bovine binucleate trophoblastic cells. *Biology of Reproduction*, **33**, 1227–1236.

Reinius, S. (1967) Ultrastructure of blastocyst attachment in the mouse. *Zeitschrift fur Zellforschung Mikroskopie Anatomie*, **77L**, 257–266.

Renegar, R.H., Southard, J.N. and Talamantes, F. (1990) Immunohistochemical colocalisation of placental lactogen II and relaxin in the golden hamster (*Mesocricetus auratus*). *Journal of Histochemistry and Cytochemistry*, **38**, 935–940.

Reng, R. (1977) Die placenta von *Microcebus murinus. Miller. Zeitschrift für Süugetierkunde*, **42**, 201–214.

Reynolds, L.P. and Redmer, D.A. (1992) Growth and microvascular development of the uterus during early pregnancy in ewes. *Biology of Reproduction*, **47**, 698–708.

Rhodes, L., Cowan, R.G., Nathanielz, P.W. and Reimers, T.J. (1986) Production of progesterone and placental lactogen by ovine binucleate trophoblast cells. *Biology of Reproduction*, **34**, Suppl. 1 (abstract no. 84).

Rice, G.E. and Thorburn, G.D. (1986) Characterisation of particle associated choriosomatomammotropin and progesterone in ovine placentomes. *Journal of Endocrinology*, **111**, 217–223.

Richart, R. (1961) Studies of placental morphology. A radioautographic study of human placenta utilising tritiated thymidine. *Proceedings of the Society for Experimental Biology and Medicine*, **106**, 829–831.

Ricketts, A.P. and Flint, A.P.F. (1980) Onset of synthesis of progesterone by ovine placenta. *Journal of Endocrinology*, **86**, 337–347.

Ricketts, A.P., Galil, A.K.A., Ackland, N., Heap, R.B. and Flint, A.P.F. (1980) Activation by corticosteroids of steroid metabolizing enzymes in ovine placental explants *in vitro*. *Journal of Endocrinology*, **85**, 457–469.

Riley, S.C., Walton, J.C., Herlick, J.M. and Challis, J.R.G. (1991) The localisation and distribution of CRH in the human placenta and fetal membranes throughout gestation. *Journal of Clinical Endocrinology and Medicine*, **72**, 1001–1007.

Roberts, R.M. (1989) Conceptus interferons and maternal recognition of pregnancy. *Biology of Reproduction*, **40**, 449–452.

Roberts, R.M. (1992) Interferons as hormones of pregnancy. *Endocrinological Reviews*, **13**, 432–442.

Roberts, R.M., Godkin, J.D., Bazer, F.W., Fincher, K.B., Thatcher, W.W., Knickerbocker, J.J. and Bartol, F.F. (1985) Antiluteolysins produced by mammalian conceptuses, in *Implantation of the Human Embryo* (eds R.G. Edwards, J.M. Purdy and P.C. Steptoe), Academic Press, London, pp. 253–273.

Roberts, R.M., Farin, C.E. and Cross, J.C. (1990) Trophoblast proteins and maternal recognition of pregnancy. *Oxford Review of Reproductive Biology*, **12**, 147–180.

Robinson, J.S., Kingston, E.J., Jones, C.T. and Thorburn, G.D. (1979) Studies on experimental growth retardation in sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. *Journal of Developmental Physiology*, **1**, 379–398.

Robertson, W.B., Khong, T.Y., Brosens, C.I., Dewolf, F., Sheppard, B.L. and Bonnar,J. (1986) The placental bed biopsy: review from three european centres. *American Journal of Obstetrics and Gynecology*, **155**, 401–412.

Rodewald, R. and Kraehenbuhl, J.P. (1984) Receptor mediated transport of IgG. *Journal of Cell Biology*, **99**, 159–164.

Rosen, S.W. (1986) New placental proteins: chemistry, physiology and clinical use. *Placenta*, **7**, 575–594.

Rossant, J. and Frels, W.I. (1980) Interspecific chimaeras in mammals: successful production of live chimaeras between *Mus musculus* and *Mus caroli*. *Science*, **208**, 419–421.

Rossman, I. (1940) The deciduomal reaction in the Rhesus monkey (*Macaca mulatta*). *American Journal of Anatomy*, **66**, 277–366.

Rowson, L.E.A. and Moor, R.M. (1966) Development of the sheep conceptus during the first 14 days. *Journal of Anatomy*, **100**, 777–785.

Rugh, R. (1975) *The Mouse: its Reproduction and Development*, Burgess Publishing, Minneapolis, USA.

Sakbun, V., Roay, E.S.C. and Bryant-Greenwood, G.D. (1987) Immunocytochemical localisation of prolactin and relaxin C peptide in human decidua and placenta. *Journal of Clinical Endocrinology and Medicine*, **65**, 339–343.

Salamonsen, L.A., Stuchbery, S.J., Ogrady, C.M., Godkin, J.D. and Finlay, J.K. (1988) Interferon α mimics effects of ovine trophoblast protein-1 on prostaglandin and protein secretion by ovine endometrial cells *in vitro*. *Journal of Endocrinology*, **117**, R1–R4.

Samuel, C.A. and Perry, J.S. (1972) The ultrastructure of pig trophoblast transplanted to an ectopic site in the uterine wall. *Journal of Anatomy*, **113**, 139–149.

Samuel, C.A., Allen, W.R. and Steven, D.H. (1974) Studies on the equine placenta. I. Development of the microcotyledons. *Journal of Reproduction and Fertility*, **41**, 441–445.

Samuel, C.A., Jack, P.M.B. and Nathanielsz, P.W. (1975) Ultrastructural studies of the rabbit placenta in the last third of gestation. *Journal of Reproduction and Fertility*, **45**, 9–14.

Samuel, C.A., Allen, W.R. and Steven, D.H. (1976) Studies on the equine placenta. II. Ultrastructure of the placental barrier. *Journal of Reproduction and Fertility*, **48**, 257–264.

Sands, J. and Dobbing, J. (1985) Continued growth and development of the third trimester human placenta. *Placenta*, **6**, 13–22.

Sasser, R.A. and Ruder, C.A. (1987) Detection of early pregnancy in domestic ruminants. *Journal of Reproduction and Fertility*, **34**, 261–271.

Sasser, R.G., Crock, J., Runder, A. and Montgomery, C.A. (1989) Characteristics of PSPB in cattle. *Journal of Reproduction Fertility*, Suppl. 37, 109–113.

Schaerer, E., Neutra, M.R., Kraehenbuhl, J.–P. (1991) Topical review: Molecular and cellular mechanisms involved in transepithelial transport. *Journal of Molecular Biology*, **123**, 93–103.

Schiebler, T.H. and Kaufmann, P. (1981) Reifeplazenta, in *Die Plazenta des Menschen*, (eds V. Becker, T.H. Schiebler and F. Kubli), Thieme, New York.

Schindler, V.F. and DeVries, U. (1987) Protein uptake and transport by trophotaeniae absorptive cells in two Goodeid embryos. *Journal of Experimental Zoology*, **241**, 17–29.

Schindler, J.F. and Hamlett, W.C. (1993) Maternal–embryonic relations in Viviparous Teleosts. *Journal of Experimental Zoology*, **266**, 378–393.

Schlafke, S. and Enders, A.C. (1975) Cellular basis of interaction between trophoblast and uterus at implantation. *Biology of Reproduction*, **12**, 41–65.

Schlafke, S., Welsh, A.O. and Enders, A.C. (1985) Penetration of the basal lamina of the uterine luminal epithelium during implantation in the rat. *Anatomical Record*, **212**, 47–56.

Schlernitzauer, D.A. and Gilbert, P.W. (1966) Placentation and associated aspects of gestation in the bonnethead shark Sphyrna tiburo. *Journal of Morphology*, **120**, 219–232.

Schonecht, P.A., Currie, W.B. and Bell, A.W. (1992) Kinetics of PL in mid and late gestation fetuses. *Journal of Endocrinology*, **133**, 95–100.

Schuhmann, R.A. (1982) Histochemical and electron microscopical studies of maternofetal circulatory units of mature human placentas. *Obstetrics and Gynecology Annual*, **11**, 1–31.

Seeburg, P.H. and Adelman, J.P. (1984) Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. *Nature (London)*, **311**, 666–668.

Shanklin, D.R. and Sibai, B.M. (1989) Ultrastructural aspects of preeclampsia. 1. Placental bed and uterine boundary vessels. *American Journal of Obstetrics and Gynecology*, **161**, 735–741.

Sharp, D.C., McDowell, K.J., Weithenauer, J. and Thatcher, W.W. (1989) The continuum of events leading to maternal recognition of pregnancy in mares. *Journal of Reproduction and Fertility*, Suppl. 37, 101–107.

Sheldrick, E.L., Ricketts, A.P. and Flint, A.P.F. (1980) Placental production of progesterone in ovariectomized goats treated with a synthetic progestagen to maintain pregnancy. *Journal of Reproduction and Fertility*, **60**, 339–348.

Sheldrick, E.L., Ricketts, A.P. and Flint, A.P.F. (1981) Placental production of 5β-pregnane-3α,20α-diol in goats. *Journal of Endocrinology*, **90**, 151–158.

Shemesh, M. (1990) Production and regulation of progesterone in bovine CL and placenta in mid and late gestation: a personal review. *Reproduction, Fertility and Development*, **2**, 129–135.

Shennan, D.B. and Boyd, C.A.R. (1987) Ion transport by the placenta: a review of membrane

transport systems. *Biochemica et Biophysica Acta*, **906**, 437–457.

Sherman, M.I. (1983) Endocrinology of rodent trophoblast cells, in *Biology of Trophoblast* (eds C. Lokeand A. Whyte), Elsevier, Amsterdam, pp. 402–460.

Sherwood, O.D. (1988) Relaxin, in *Physiology of Reproduction*, Chapter 16 (eds E. Knobil and J. Neill), Raven Press, New York.

Shimizu, T., Tsutsui, T. and Orima, H. (1990) Incidence of transuterine migration of embryos in the dog. *Japanese Journal of Veterinary Science*, **52**, 1273–1275.

Shine, J., Seeburg, P.H., Martial, J.A., Baxter, J.D. and Goodman, H. (1977) Construction and analysis of 'recombinant DNA for human chorionic somatomammotropin'. *Nature (London)*, **270**, 494–499.

Shine, R. and Bull, J.J. (1979) The evolution of live bearing in lizards and snakes. *American Naturalist*, **113**, 905–923.

Shine, R. and Guillette, L.J. (1988) The evolution of viviparity in reptiles; a physiological model and its ecological consequences. *Journal of Theoretical Biology*, **132**, 43–50.

Sibley, C.P. and Boyd, R.D.H. (1988) Control of transfer across the mature placenta. *Oxford Reviews of Reproductive Biology*, **10**, 382–435.

Siler-Khodr, T.M. (1983) Hypothalamic-like releasing hormones of the placenta. *Clinics in Perinatology*, **10**, 553–566.

Siler-Khodr, T.M. and Khodr, G.S. (1978) Content of lutenizing hormone releasing factor in the human placenta. *American Journal of Obstetrics and Gynecology*, **130**, 216–219.

Silver, M. and Steven, D. (1975) Placental exchange of blood gases, in *Comparative Placentation* (ed. D. Steven), Academic Press, London, pp.161–188.

Silver, M., Steven, D.H. and Comline, R.S. (1973) Placental exchange and morphology in ruminants and the mare, in *Foetal and Neonatal Physiology* (eds R.S. Comline, K.W. Cross, G.S. Dawes and P.W. Nathanielz), Cambridge University Press, Cambridge, pp. 245–271.

Silver, M., Barnes, R.J., Comline, R.S. and Burton, G.J. (1982) Placental blood flow: some fetal and maternal cardiovascular adjustments during gestation. *Journal of Reproduction and Fertility*, Suppl. 31, 139–160.

Simpson, R.A., Mayhew, T.M. and Barnes, P.R. (1992) From 13 weeks to term the trophoblast of the human placenta grows by the continuous recruitment of new proliferative units: A study of nuclear number using the disector. *Placenta*, **13**, 501–512.

Sinha, A.A. and Erickson, A.W. (1974) Ultrastructure of the placenta of the Antarctic seals during the first third of pregnancy. *American Journal of Anatomy*, **141**, 263–280.

Sinha, A.A., Seal, U.S., Erickson, A.W. and Mossman, H.W. (1969) Morphogenesis of the fetal membranes of the white tailed deer. *American Journal of Anatomy*, **126**, 201–242.

Sinowatz, F. and Friess, A.E. (1983) Uterine glands of the pig during pregnancy. An ultrastructural and cytochemical study. *Anatomy and Embryology*, **166**, 121–134.

Skolek-Winnisch, R., Lipp, W., Sinowatz, F. and Friess, A.E. (1985) Enzymhistochemische untersuchungen an der Schweine placenta. II. Histotopik von Enzymen in den areolaren placenta epithelien. *Acta Histochemica*, **76**, 131–143.

Smith, C.A. and Moore, H.D.M. (1990) An ultrastructural study of early chorionic villus formation in the marmoset monkey (*Callithrix jacchus*). *Anatomy and Embryology*, **181**, 59–66.

Smith, C.A., Moore, H.D.M. and Hearn, J.P. (1987) The ultrastructure of early implantation in the marmoset monkey (*Callithrix jacchus*). *Anatomy and Embryology*, **175**, 399–410.

Smith, C.E., Cullen, W.C. and Godkin, J.D. (1990) Ultrastructural morphometric analysis of the uterine epithelium during early pregnancy in the sheep. *Journal of Reproduction and Fertility*, **89**, 517–525.

Snow, M.A.L. and Ansell, J.D. (1974) The chromosomes of giant trophoblast cells of the mouse. *Proceedings of the Royal Society*, **B 187**, 93–98.

Soares, M.J. (1987) Developmental changes in the intraplacental distribution of PL and alkaline phosphatase in the rat. *Journal of Reproduction and Fertility*, **79**, 93–98.

Soares, M.J., Colosi, P., Ogren, L. and Talamantes, F. (1983) Identification and partial characterisation of a lactogen from the midpregnant mouse conceptus. *Endocrinology*, **112**, 1313–1317.

Soares, M.J., Faria, T.N., Roby, K.F. and Deb, S. (1991) Pregnancy and the PRL family of hormones: coordination of anterior pituitary, uterine and placental expression. *Endocrinological Reviews*, **12**, 402–423.

Sooranna, S.R. and Contractor, S.F. (1991) Vectorial transcytosis of IgG by human term

trophoblast cells in culture. *Experimental Cell Research*, **192**, 41–46.

Southard, J.N. and Talamantes, F. (1991) Placental PRL-like proteins in rodents: variations on a structural theme. *Molecular and Cellular Endocrinology*, **79**, C133–C140.

Sparks, J.W., Lynch, A. and Glinsmann, W.H. (1976) Regulation of rat liver glycogen synthesis and activities of glycogen cycle enzymes by glucose and galactose. *Metabolism*, **25**, 47–55.

Starck, D. (1949) Einbeitrag zur Kenntnis der Placentation bei der Macrosceliden. *Zeitschrift fur Anatomie Entwicklungs Geschichte*, **114**, 319–339.

Steele, P.A., Flint, A.P.F. and Turnbull, A.C. (1976) Activity of steroid C-17,20 lyase in the ovine placenta: effect of exposure to foetal glucocorticoid. *Journal of Endocrinology*, **69**, 239–246.

Steer, H. (1971) Implantation of the rabbit blastocyst: the adhesive phase of implantation. *Journal of Anatomy*, **109**, 215–227.

Stegemann, J.H.J. (1974) Placental development in the sheep and its relation to fetal development. A qualitative and quantitative anatomic and histologic study. *Bijdragen tot de Dierkunde (Contributions to Zoology)*, **44**, 3–72.

Stegink, L.D., Pitkin, R.M., Reynolds, W.A., Filer, L.J., Boaz, D.P. and Brummel,A.C. (1975) Placental transfer of glutamate and its metabolite in the primate. *American Journal of Obstetrics and Gynecology*, **122**, 170.

Stegink, L.D., Pitkin, R.M., Reynolds, W.A., Brummel, M.C. and Filer, L.J. (1979) Placental transfer of aspartate and its metabolites in the primate. *Metabolism*, **28**, 669–676.

Steinman, R.M., Mellman, I.S., Muller, W.A. and Cohn, Z.A. (1983) Endocytosis and the recycling of the plasma membrane. *Journal of Cell Biology*, **96**, 1–27.

Stephens, R.J. (1962) Histology and histochemistry of the placenta and fetal membranes of the bat Tadarida brasiliensis cynocephala. *American Journal of Anatomy*, **111**, 239–285.

Stephens, R.J. (1969) The development and fine structure of the allantoic placental barrier in the bat Tadarida brasiliensis cynocephala. *Journal of Ultrastructure Research*, **28**, 371–398.

Stephens, R.J. and Cabral, L. (1971) Direct contribution of the cytotrophoblast to the syncytiotrophoblast in the diffuse labyrinthine endotheliochorial placenta of the bat. *Anatomical Record*, **169**, 243–252.

Stephens, R.J. and Cabral, L.J. (1972) The diffuse labyrinthine endotheliodichorial placenta of the free tailed bat: a light and electron microscopy study. *Anatomical Record*, **172**, 221–252.

Steven, D.H. (1966) Further observations on placental circulation in the sheep. *Journal of Physiology, London*, **187**, 18P–19P.

Steven, D.H. (ed.) (1975a) *Comparative Placentation*, Academic Press, New York.

Steven, D.H. (1975b) Separation of the placenta in the ewe: an ultrastructural study. *Quarterly Journal of Experimental Physiology*, **60**, 37–44.

Steven, D.H. (1982) Placentation in the mare. *Journal of Reproduction and Fertility*, **31**, 41–55.

Steven, D.H. (1983) Interspecies differences in the structure and function of trophoblast, in *Biology of Trophoblast*, (eds C. Loke and A. Whyte), Elsevier, Amsterdam, pp. 111–136.

Steven, D.H. and Samuel, C.A. (1975) Anatomy of the placental barrier in the mare. *Journal of Reproduction and Fertility*, Suppl. 23, 579–582.

Steven, D.H., Bass, F., Jansen, C.J.M., Krane, E.J., Mallon, K., Samuel, C.A., Thomas, A.L. and Nathanielz, P.W. (1978) Ultrastructural changes in the placenta of the ewe after fetal pituitary stalk section. *Quarterly Journal of Experimental Physiology*, **634**, 221–231.

Steven, D.H., Jeffcoat, L.B., Mallon, K.A., Ricketts, S.W., Rossdale, P.D. and Samuel, C.A. (1979) Ultrastructural studies of the equine uterus and placenta following parturition. *Journal of Reproduction and Fertility*, **17**, 579–586.

Steven, D.H., Burton, G.J., Kumar, J. and Nathanielsz, P.W. (1980a) Ultrastructural observations on the placenta of the alpaca (*Lama pacos*). *Placenta*, **1**, 21–32.

Steven, D.H., Mallon, K.A. and Nathanielsz, P.W. (1980b) Sheep trophoblast in monolayer culture. *Placenta*, **1**, 209–221.

Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992) Blastocyst implantation depends upon maternal expression of LIF. *Nature*, **359**, 76–78.

Stewart, H.J., Jones, D.S.C., Pascall, J.C., Popkin, R.M. and Flint, A.P.F. (1988) The contributions of recombinant DNA techniques to reproductive biology. *Journal of Reproduction and Fertility*, **83**, 1–57.

Stewart, H.J., McCann, S.H.E., Barker, P.J., Lee, K.E., Lamming, G.E. and Flint, A.P.F. (1987) Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. *Journal of Endocrinology*, **115**, R13–R15.

Stewart, I. (1984) A morphological study of granulated metrial gland cells and trophoblast cells in the labyrinthine placenta of the mouse. *Journal of Anatomy*, **139**, 627–638.

Stewart, I. (1985) Granulated metrial gland cells in the lungs of mice in pregnancy and pseudopregnancy. *Journal of Anatomy*, **140**, 551–563.

Stewart, I. and Peel, S. (1977) The structure and differentiation of granulated metrial gland cells of the pregnant mouse uterus. *Cell and Tissue Research*, **184**, 517–527.

Stewart, J.R. (1993) Yolk sac placentation in reptiles: structural innovation in a fundamental vertebrate fetal nutritional system. *Journal of Experimental Zoology*, **266**, 431–449.

Stewart, J.R. and Blackburn, D.G. (1988) Reptilian placentation: structural diversity and terminology. *Copeia*, **4**, 839–852.

Strain, A.J., Hill, D.J., Swenne, I. and Milner, R.D.G. (1987) Regulation of DNA synthesis in human fetal hepatocytes by PL, GH, and insulin like growth factor I/somatomedin C. *Journal of Cellular Physiology*, **132**, 33–40.

Strickland, S. and Richards, W.G. (1992) Invasion of the trophoblasts. *Cell*, **71**, 355–357.

Stroband, H.W.J., Taverne, N., Largenfeld, K. and Barends, P.M.G. (1986) The ultrastructure of the uterine epithelium of the pig during the estrous cycle and early pregnancy. *Cell and Tissue Research*, **246**, 81–89.

Stulc, J. (1989) Extracellular transport pathways in the hemochorial placenta. *Placenta*, **10**, 113–119.

Sturgess, I. (1948) The early embryology and placenta of Procavia capensis (*Hyrax*). *Acta Zoologica*, **29**, 393–479.

Summers, P.M., Shephard, A.M., Hodges, J.K., Kydd, J., Boyle, M.S. and Allen, W.R. (1987) Successful transfer of the embryos of Przewalski's horses (*Equus przewalskii*) and Grant's zebra (*E. birchelli*) to domestic mares (*E. caballus*). *Journal of Reproduction and Fertility*, **80**, 13–20.

Tachi, S. and Tachi, C. (1979) Ultrastructural studies on maternal embryonic cell interactions during experimentally induced implantation of rat to the endometrium of the mouse. *Developmental Biology*, **68**, 203–223.

Tait, A.D., Hodge, L.C. and Allen, W.R. (1982) Production of an equilin precursor by the fetal horse gonad. *ICRS Medical Science*, **10**, 346–347.

Talamantes, F. and Ogren, L. (1988) The placenta as an endocrine organ: Polypeptides, in *Physiology of Reproduction*, Chapter 52 (eds E. Knobil and J. Neill), Raven Press, New York.

Tan, L. and Rousseau, P. (1983) The chemical identity of the immunoreactive LHRH-like peptide biosynthesized in the human placenta. *Biochemical and Biophysical Research Communications*, **109**, 1061–1071.

Tao, T.W. and Hertig, A.T. (1965) Viability and differentiation of human trophoblast in organ culture. *American Journal of Anatomy*, **116**, 315–328.

Tarachand, U. (1986) Decidualisation: origin and role of the associated cells. *Biologie Cellulaire*, **57**, 9–16.

Tatarinov, Y.S. and Sokolov, A.V. (1977) Development of a radioimmunoassay for pregnancy-specific β-globulin and its measurement in serum of patients with trophoblastic and nontrophoblastic tumours. *International Journal of Cancer*, **19**, 161–166.

Tavolga, W.N. and Rugh, R. (1947) Development of the platyfish Platypoecilus maculatus. *Zoologica*, **32**, 1–16.

Tawfik, O.W., Hunt, D.S. and Wood, G.W. (1986) Implication of PGE_2 in soluble factor mediated immune suppression by murine decidual cells. *American Journal of Reproduction and Immunological Microbiology*, **12**, 111–117.

Taylor, M.J., Webb, R., Mitchell, M.D. and Robinson, J.S. (1982) Effect of progesterone withdrawal in sheep during late pregnancy. *Journal of Endocrinology*, **92**, 85–93.

Taylor, M.J., McMillan, K., Jenkin, G., Robinson, J.S. and Thorburn, G.D. (1983) The regulation of OPL, the role of the fetal hypothalamo-pituitary axis. *Journal of Developmental Physiology*, **5**, 251–258.

Teasdale, F. (1976) Numerical density of nuclei in the sheep placenta. *Anatomical Record*, **185**, 187–196.

Tedde, G. and Tedde-Piras, A. (1978) Mitotic index of the Langhans cells in the normal human placenta from the early stages of pregnancy to term. *Acta Anatomica*, **100**, 114–119.

Thorburn, G.D. (1991) The placenta, prostaglandins and parturition, a review. *Reproduction, Fertility and Development*, **3**, 277–294.

Thorburn, G.D. and Mattner, P.E. (1971) Anastomosis of the utero-ovarian and anterior mammary veins for collection of utero-ovarian venous blood: progesterone secretion rates in cyclic ewes. *Journal of Endocrinology*, **50**, 307–320.

Thordarson, G., McDowell, G.H., Smith, S.V.,

Iley, S. and Forsyth, I.A. (1987) Effects of continuous i.v. infusion of an ovine placental extract enriched in PL on plasma hormones, metabolites and metabolic biokinetics in non pregnant sheep. *Journal of Endocrinology*, **113**, 277–283.

Thornburg, K.L., Bissonnette, J.M. and Faber, J.J. (1976) Absence of fetal placental waterfall phenomenon in chronically prepared fetal lambs. *American Journal of Physiology*, **230**, 886–892.

Tiedemann, K. and Minuth, W.W. (1980) The pig yolk sac: fine structure of the posthematopoietic organ. *Histochemistry*, **68**, 133–146.

Toth, M., Asboth, G. and Hertelendy, F. (1983) Turnover of lipid bound arachidonic acid and biosynthesis of prostanoids in the endometrium and myometrium of the laying hen. *Archives of Biochemistry and Biophysics*, **226**, 27–36.

Towler, C.M., Horne, C.H.W., Jandial, V., Campbell, D.M. and MacGillivray, I. (1976) Plasma levels of pregnancy-specific β-glycoprotein in normal pregnancy. *British Journal of Obstetrics and Gynaecology*, **83**, 775–779.

Tsoulos, N.G., Colwell, J.R., Battaglia, F.C., Makowski, E.L. and Meschia, G. (1971) Comparison of glucose, fructose and oxygen uptakes by fetuses of fed and starved ewes. *American Journal of Physiology*, **221**, 234–237.

Tsutsumi, Y. (1962) The vascular pattern of the placenta in farm animals. *Journal of the Faculty of Agriculture Hökkaido University Sapporo*, **52**, 372–482.

Turner, C.L. (1947) Viviparity in the teleost fishes. *Science Monthly*, **65**, 508–518.

Tyndale-Biscoe, H. and Renfree, M. (1987) *Reproductive Physiology of Marsupials*, Cambridge University Press.

Ullman, M.B. and Reimers, T.J. (1989) Progesterone production by binucleate trophoblastic cells of cows. *Journal of Reproduction and Fertility*, Suppl. 37, 173–179.

Ulysses, S.S., Akhouri, A., Doe, S. and Doe, R.P. (1972) Placental iron transfer: relationship to placental anatomy and phylogeny of the mammals. *American Journal of Anatomy*, **134**, 263–269.

Vanderpuye, O.A. and Smith, C.A. (1987) Proteins of the apical and basal plasma membranes of the human placental syncytium: immunochemical and electrophoretic studies. *Placenta*, **8**, 591–608.

Van Duyne, C.M., Parker, H.R., Havel, R.J. and Holm, L.M. (1960) Free fatty acid metabolism in fetal and newborn sheep. *American Journal of Physiology*, **199**, 987–990.

Van Wyk, L.C., Van Niekerk, C.H. and Belonje, P.C. (1972) Evolution of the post partum uterus of the ewe. *Journal of South African Veterinary Association*, **43**, 19–26.

Varmuza, S., Prideaux, V., Kothary, R. and Rossant, J. (1988) Polytene chromosomes in mouse trophoblast giant cells. *Development*, **102**, 127–134.

Venuto, R.C., Cox, J.W., Stein, J.H. and Ferris, T.F. (1976) The effect of changes in perfusion pressure on uteroplacental blood flow in the pregnant rabbit. *Journal of Clinical Investigation*, **57**, 938–944.

Verstegen, J., Fellmann, D. and Beckers, J.F. (1985) Immunodetection of the bovine chorionic somatomammotropin. *Acta Endocrinologia*, **109**, 403–410.

Wadsworth, P.F., Lewis, D.J. and Heywood, R. (1980) The ultrastructural features of progestagen induced decidual cells in the rhesus monkey (*Macaca mulatta*). *Contraception*, **22**, 189–198.

Wagner, W.C. and Hansel, W. (1969) Reproductive physiology of the post partum cow. 1. Clinical and histological findings. *Journal of Reproduction and Fertility*, **18**, 493–500.

Wales, R.G. and Cuneo, C.L. (1989) Morphological and chemical analysis of the sheep conceptus from the 13th to the 19th day of pregnancy. *Reproduction Fertility and Development*, **1**, 31–39.

Wallace, C.R., Collier, R.J., Bott, D.J, Byatt, J.C. and Bremel, R.D. (1985) Bovine placental lactogen concentrations in maternal and fetal fluids. *Journal of Animal Science*, **61**, Suppl. 1, Abstract No. 377.

Wallenburg, H.C.S. (1981) Modulation and regulation of uteroplacental blood flow. *Placenta*, Suppl. 1, 45–64.

Walls, E.W. (1938) Myrmecophaga jubata: an embryo with a placenta. *Journal of Anatomy*, **73**, 311–317.

Wango, E.O., Wooding, F.B.P. and Heap, R.B. (1990a) The role of trophoblastic cells in implantation in the goat: a morphological study. *Journal of Anatomy*, **171**, 241–257.

Wango, E.O., Wooding, F.B.P. and Heap, R.B. (1990b) The role of trophoblastic cells in implantation in the goat: a quantitive study. *Placenta*, **11**, 381–394.

Wango, E.O., Wooding, F.B.P. and Heap, R.B. (1991) Progesterone and 5β pregnanediol pro-

duction by isolated fetal binucleate cells from sheep and goats. *Journal of Endocrinology*, **129**, 283–289.

Waters, M.J., Oddy, V.H., McCloghny, C.E., Gluckman, P.D., Duplock, R., Owens,P.C. and Brinsmead, M.W. (1985) An examination of the proposed roles of placental lactogen in the ewe by means of antibody neutralisation. *Journal of Endocrinology*, **106**, 377–386.

Wathes, D.C. (1992) Commentary: Embryonic mortality and the uterine environment. *Journal of Endocrinology*, **134**, 321–325.

Wathes, D.C. and Wooding, F.B.P. (1980) An electron microscopic study of implantation in the cow. *American Journal of Anatomy*, **159**, 285–306.

Watkins, W.B. and Reddy, S. (1980) Ovine placental lactogen in the cotyledonary and intercotyledonary placenta of the ewe. *Journal of Reproduction and Fertility*, **58**, 411–414.

Weekes, H.L.W. (1935) A review of the placentation among reptiles with particular regard to the function and evolution of the placenta. *Proceedings of the Herpetological Society*, **Part 2**, 625–645.

Wehrenberg, W.B., Chaichareon, D.P., Dierschke, D.J., Rankin, J.H. and Ginther,O.J. (1977) Vascular dynamics of the reproductive tract in the female rhesus monkey: relative contributions of ovarian and uterine arteries. *Biology of Reproduction*, **17**, 148–153.

Welsh, A.O. and Enders, A.C. (1983) Occlusion and reformation of rat uterine epithelium during pregnancy. *American Journal of Anatomy*, **167**, 463–477.

Welsh, A.O. and Enders, A.C. (1985) A light and electron microscopic examination of the mature decidual cells of the rat with emphasis on the antimesometrial decidua and its degeneration. *American Journal of Anatomy*, **172**, 1–30.

Welsh, A.O. and Enders, A.C. (1987) Trophoblast decidual cell interactions and establishment of maternal blood circulation in the parietal yolk sac placenta of the rat. *Anatomical Record*, **217**, 203–219.

Welsh, A.O. and Enders, A.C. (1991a) Chorioallantoic placental formation in the rat. I. Luminal epithelial cell death and ECM modifications in the mesometrial region of the implantation chamber. *American Journal of Anatomy*, **192**, 215–231.

Welsh, A.O. and Enders, A.C. (1991b) Chorioallantoic placental formation in the rat. II. Angiogenesis and maternal blood circulation in the mesometrial region of the implantation chamber prior to placental formation. *American Journal of Anatomy*, **192**, 346–365.

Wewer, V.M., Faber, M., Liotta, L.A. and Albrechtsen, R. (1985) Immunochemical and ultrastructural assessment of the nature of the pericellular basement membrane of human decidual cells. *Laboratory Investigation*, **53**, 624–633.

Whitwell, K.E. and Jeffcott, L.B. (1975) Morphological studies on the fetal membranes of the normal singleton foal at term. *Research in Veterinary Science*, **19**, 44–55.

Whyte, A. and Allen, W.R. (1985) Equine endometrium at preimplantation stages of pregnancy has specific glycosylated regions. *Placenta*, **6**, 537–542.

Wichtrup, L.G. and Greven, H. (1985) Uptake of ferritin by the trophotaeniae of the Goodeid Fish Ameca splendens Miller and Fitzsimons 1971 (*Teleostei; Cyprinodontiformes*). *Cytobiology*, **42**, 33–40.

Wigglesworth, J.S. (1969) Vascular anatomy of the human placenta and its significance for placental pathology. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **76**, 979–989.

Wigmore, P.M.C. and Strickland, N.C. (1985) Placental growth in the pig. *Anatomy and Embryology*, **173**, 263–268.

Wild, A.E. (1981) Endocytotic mechanisms in protein transfer across the placenta. *Placenta*, Suppl. 1, 165–186.

Wiley, A.A., Baktol, F.F. and Barron, D.H. (1987) Histogenesis of the ovine uterus. *Journal of Animal Science*, **64**, 1262–1269.

Wilkening, R.B. and Meschia, G. (1992) Current topic: Comparative physiology of placental oxygen transport. *Placenta*, **13**, 1–15.

Willingham, M.C. and Pastan, I. (1984) Endocytosis and exocytosis: current concepts of vesicle traffic in animal cells. *International Review Cytology*, **92**, 51-86.

Wilmut, I., Sales, D.I. and Ashworth, C.J. (1986) Maternal and embryonic factors associated with prenatal loss in mammals. *Journal of Reproduction and Fertility*, **76**, 851–864.

Wimsatt, W.A. (1951) Observations on the morphogenesis cytochemistry and significance of the binucleate giant cells of the placenta of ruminants. *American Journal of Anatomy*, **89**, 233–282.

Wimsatt, W.A. (1974) Morphogenesis of the fetal membranes and placenta of the black bear Ursus

americanus (*Pallas*). *American Journal of Anatomy*, **140**, 471–496.

Wimsatt, W.A. and Enders, A.C. (1980) Structure and morphology of uterus, placenta and paraplacental organs of the neotropical disc winged bat Thyroptera tricolor spix (*Microchiroptera: Thyropteridae*). *American Journal of Anatomy*, **159**, 209–243.

Wimsatt, W.A., Enders, A.C. and Mossman, H.W. (1973) A re-examination of the chorioallantoic placental membrane of a shrew, Blarina brevicauda: resolution of a controversy. *American Journal of Anatomy*, **138**, 207–234.

Wintenberger-Torres, S. and Flechon, J. (1974) Ultrastructural evolution of the trophoblast cells in the preimplantation sheep blastocyst from day 8 to day 18. *Journal of Anatomy*, **118**, 143–153.

Wislocki, G.B. (1929) On the placentation of primates with a consideration of the phylogeny of the placenta. *Carnegie Institute Contributions to Embryology*, **20**, 51–80.

Wislocki, G.B. (1930) A series of placental stages of a platyrrhine monkey Ateles geoffroyi, with some remarks on age, sex and breeding period in platyrrhines. *Carnegie Contributions to Embryology*, **22**, 173–200.

Wislocki, G.B. and Amoroso, E.C. (1956) The placenta of the Wolverine (*Gulo gulo luscus*). *Bulletin of the Museum of Comparative Zoology, Harvard University*, **114**, 91–100.

Wislocki, G.B. and Dempsey, E.W. (1946) Histochemical reactions in the placenta of the cat. *American Journal of Anatomy*, **78**, 1–46.

Wislocki, G.B. and Streeter, G.L. (1938) On the placentation of the Macaque (Macaca mulatta) from the time of implantation until the formation of the definitive placenta. *Carnegie Institute Contributions to Embryology*, **27**, 1–66.

Wislocki, G.B. and Westhuysen, O.P.V. (1940) The placentation of Procavia capensis, with a discussion of the placental affinities of the Hyracoides. *Contributions to Embryology*, **27**, 67–88.

Wooding, F.B.P. (1980) Electron microscopic localisation of binucleate cells in the sheep placenta using phosphotungstic acid. *Biology of Reproduction*, **22**, 357–365.

Wooding, F.B.P. (1981) Localisation of ovine placental lactogen in sheep placentomes by electron microcope immunocytochemistry. *Journal of Reproduction and Fertility*, **62**, 15–19.

Wooding, F.B.P. (1982a) Structure and function of placental binucleate (giant) cells. *Bibliotheca Anatomica*, **22**, 134–139.

Wooding, F.B.P. (1982b) The role of the binucleate cell in ruminant placental structure. *Journal of Reproduction and Fertility*, Suppl. 31, 31–39.

Wooding, F.B.P. (1983) Frequency and localisation of binucleate cells in the placentomes of ruminants. *Placenta*, **4**, 527–540.

Wooding, F.B.P. (1984) Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep. *American Journal of Anatomy*, **170**, 233–250.

Wooding, F.B.P. (1987) Ultrastructural evidence for placental lactogen transport and secretion in ruminants. *Journal of Physiology*, **386**, 26P.

Wooding, F.B.P. (1992) The synepitheliochorial placenta of ruminants: Binucleate cell fusions and hormone production. *Placenta*, **13**, 101–113.

Wooding, F.B.P. and Beckers, J.F. (1987) Trinucleate cells and the ultrastructural localisation of bovine placental lactogen. *Cell and Tissue Research*, **247**, 667–673.

Wooding, F.B.P. and Staples, L.D. (1981) Functions of the trophoblast papillae and binucleate cells in implantation in the sheep. *Journal of Anatomy*, **133**, 110–112.

Wooding, F.B.P. and Wathes, D.C. (1980) Binucleate cell migration in the bovine placentome. *Journal of Reproduction and Fertility*, **59**, 425–430.

Wooding, F.B.P., Chambers, S.G., Perry, J.S., George, M. and Heap, R.B. (1980) Migration of binucleate cells in the sheep placenta during normal pregnancy. *Anatomy and Embryology*, **158**, 361–370.

Wooding, F.B.P., Flint, A.P.F., Heap, R.B. and Hobbs, T. (1981) Autoradiographic evidence for migration and fusion of cells in the sheep placenta; resolution of a problem in placental classification. *Cell Biology International Reports*, **5**, 821–827.

Wooding, F.B.P., Staples, L.D. and Peacock, M.A.P. (1982) Structure of trophoblast papillae on the sheep conceptus at implantation. *Journal of Anatomy*, **134**, 507–516.

Wooding, F.B.P., Flint, A.P.F., Heap, R.B., Morgan, G., Buttle, H.L. and Young, I.R. (1986) Control of binucleate cell migration in the placenta of ruminants. *Journal of Reproduction and Fertility*, **76**, 499–512.

Wooding, F.B.P., Morgan, G., Forsyth, I.A., Butcher, G., Hutchings, A., Billingsley, S.A.

and Gluckman, P.D. (1992) Light and electron-microscopic studies of cellular localisation of OPL with monoclonal and polyclonal antibodies. *Journal of Histochemistry and Cytochemistry*, **40**, 1001–1009.

Wooding, F.B.P., Hobbs, T., Morgan, G., Heap, R.B. and Flint, A.P.F. (1993) Cellular dynamics of growth in sheep and goat synepitheliochorial placentomes: An autoradiographic study. *Journal of Reproduction and Fertility*, **98**, 275–283.

Woollacott, R.M. and Zimmer, R.L. (1975) A simplified placenta-like system for the transport of extraembryonic nutrients during embryogenesis of Rugula neritina (*Bryozoa*). *Journal of Morphology*, **147**, 355–378.

Wourms, J.P. (1981) Viviparity: the maternal fetal relationship in fishes. *American Zoologist*, **21**, 473–515.

Wu, M.C., Chen, Z.Y., Jarrell, V.L. and Dzuik, P.J. (1989) Effect of initial length of uterus per embryo on fetal survival and development in the pig. *Journal of Animal Science*, **67**, 1764–1772.

Wu, W.X., Brooks, J., Millar, M.R., Ledger, W.L., Saunders, P.T.K., Glasier, A.F. and McNeilly, A.S. (1991) Localisation of the sites of synthesis and action of PRL by immunocytochemistry and in situ hybridisation within the human uteroplacental unit. *Journal of Molecular Endocrinology*, **7**, 241–247.

Wynn, R.M. (1967) Comparative electron microscopy of placental junctional zone. *American Journal of Obstetrics and Gynecology*, **19**, 644–661.

Wynn, R.M. (1974) Ultrastructural development of the human decidua. *American Journal of Obstetrics and Gynecology*, **118**, 652–670.

Wynn, R.M., Hoschner, J.A. and Oduor-Okelo, D. (1990) The interhemal membrane of the spotted hyena: an immunohistological reappraisal. *Placenta*, **11**, 215–221.

Xavier, F. (1976) An exceptional reproductive strategy in Anura: Nectophrynoides occidentalis Angel (*Bufonidae*), in *Major Patterns in Vertebrate Evolution* (eds M.K. Hecht, B.M. Hecht and P.C. Goody), Plenum, New York.

Xuan, W.W., Millar, M., Brooks, J., Saunders, P., Ledger, W., Glasier, A. and McNeilly, A.S. (1990) Localisation of the sites of synthesis and action of PRL by immunocytochemistry and in situ hybridisation within the human fetoplacental unit. *Journal of Endocrinology*, **127**, Supplement, Abstract 126.

Yaron, Z. (1977) Embryo maternal interactions in the lizard Xantusia vigilis, in *Reproduction and Evolution* (eds J.H. Calaby and C.H. Tyndale-Biscoe) Australian Academy of Science, Canberra.

Yamaguchi, M., Ogren, L., Endo, H., Thordarson, G., Bigsby, R.M. and Talamantes, F. (1992) Production of mPL I and II by the same giant cell. *Endocrinology*, **131**, 1595–1602.

Young, M. (1981) Placental amino acid transfer and metabolism. *Placenta*, Suppl. 2, 177–184.

Young, M. (1988) Handling of amino acids by the fetoplacental unit, in *Placental Nutrition* (ed. B.S. Lindblad), Academic Press, New York, pp. 25–43.

Zavy, M.T., Bazer, F.W., Sharp, D.C. and Wilcox, C.J. (1979) Uterine luminal proteins in the cycling mare. *Biology of Reproduction*, **20**, 689–698.

Zavy, M.T., Sharp, D.C., Bazer, F.W., Fazleabas, A., Sessions, F. and Roberts, R.M. (1984) Identification of stage specific and hormone induced polypeptides in the uterine secretions of the mare during the oestrous cycle and pregnancy. *Journal of Reproduction and Fertility*, **64**, 199–207.

Zhou, J. and Bondy, C. (1992) IGF II and its binding proteins in placental development. *Endocrinology*, **131**, 1230–1240.

Zoli, A.P., Demez, P., Beckers, J.F., Reznik, M. and Beckers, A. (1992) Light and electronmicroscopic immunolocalisation of bPAG in the bovine placentome. *Biology of Reproduction*, **46**, 623–629.

HORMONES OF THE PLACENTA: hCG AND hPL

5

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5.1 INTRODUCTION

The pre-eminent function of the human placenta is transfer of nutrients, gases and waste products between the mother and the fetus. At the same time, it is also extraordinarily active in the synthesis of a wide range of proteins and other molecules. Some of these are the structural proteins, enzymes, etc., which are part of the normal functional equipment of cells throughout the body and thus are in no sense specific to this particular organ. The respiratory enzymes are one example. Another example, though slightly more limited in overall distribution, is the group of enzymes responsible for steroid synthesis and interconversion; these do not differ from their counterparts in other steroid-producing tissues. In addition, the placenta contains low concentrations of ACTH-related peptides and hypothalamic releasing hormones, none of which differs significantly from their hypothalamic–pituitary counterparts (J.N. Lee *et al.*, 1981, 1982).

More important, and the theme of the present chapter, are the two most familiar members of the group of proteins which the placenta undoubtedly synthesizes and secretes, and which in the past have frequently been described as 'specific' to this organ: human chorionic gonadotrophin (hCG) and human placental lactogen (hPL). However, it is now apparent that there is no material which is qualitatively specific in the sense of occurring uniquely in the placenta and not in any other site. Thus, most (possibly all) of the classical products of the human placenta, including hCG and hPL, can be found in seminal fluid and ovarian follicular fluid and small amounts of hCG have been recognized in the pituitary gland and many normal tissues (sections 5.3.1 and 5.4.1). This chapter is concerned with the two classical protein hormones of group 1, hCG and hPL.

5.2 HUMAN CHORIONIC GONADOTROPHIN

5.2.1 HISTORICAL SURVEY

The hormones of the placenta have only been studied in detail in the last 60 years, but certain properties of placental tissue were noted much earlier. Medvei (1982) described the use of dried placentas to improve the fertility of Chinese and Javanese women. In parts of Hungary it was the custom for women wishing to limit their families to burn their placentas and mix the ashes in the drink of their husbands. Furthermore, it has long been observed that some female animals eat their placentas and that chimpanzees in labour drink their own urine.

The human placenta was first shown to produce a gonadotrophin by Hirose in 1919 (quoted by Hussa, 1987). Human placental tissue was implanted under the skin of rabbits and morphological changes associated with ovulation and fertilization were observed in the ovaries, uterus and vagina. Similar changes were noted by Aschheim and Zondek (1927a), when they implanted 'hypophyseal' (anterior pituitary) tissue under the skin of immature mice. This resulted in the rapid onset of oestrus as a result of ovarian maturation (quoted by Aschheim, 1930). Aschheim and Zondek (1927b) subsequently discovered that early pregnancy sera and urine contained extremely high levels of this gonadotrophic substance, which they referred to as 'Hypophysenvorderlappenhormons' (hypophyseal hormone). However, the hormone was found at high levels in, and was therefore probably produced by, the placenta and decidua. They concluded that the uterine changes were a result of ovarian follicular maturation and thus indirect effects of the hormone. The morphological changes in the ovaries (multiple follicular growth, rupture and corpus luteum formation) formed the basis for the Aschheim–Zondek bioassay

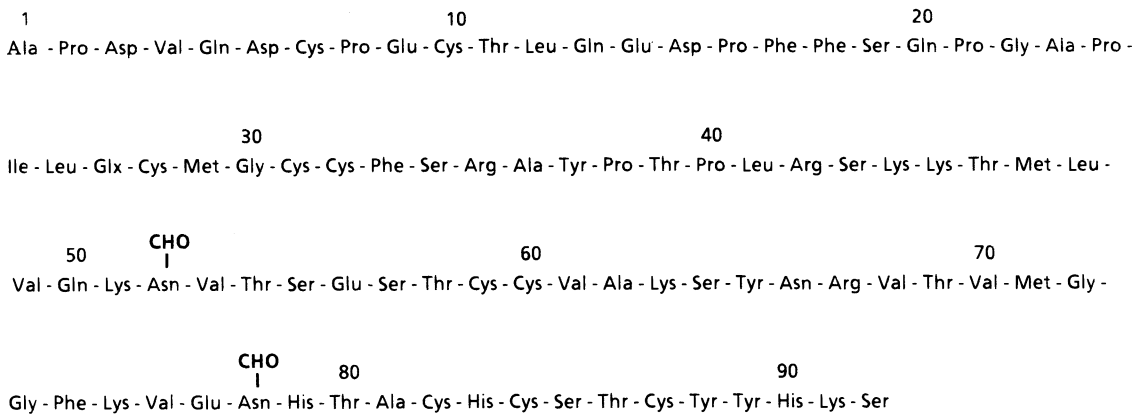

Figure 5.1 Amino acid sequence of the glycoprotein hormone α-subunit. CHO indicates positions of glycosylation. (Adapted from Hussa, 1987.)

for the early diagnosis of pregnancy using a specimen of urine (Aschheim and Zondek, 1927a).

5.3 CHORIONIC GONADOTROPHIN AND THE GLYCOPROTEIN HORMONES

Chorionic gonadotrophin (CG) is a member of a group of four structurally homologous proteins commonly referred to as the glycoprotein hormones. As the name implies, the hormone protein chains are glycosylated; hCG contains approximately 30% carbohydrate by weight. The other members are luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). CG differs from the other glycoprotein hormones in that it is produced by the placenta whilst LH, FSH and TSH are pituitary products. CG, LH and FSH are gonadotrophins, stimulating ovarian and testicular functions via regulation of gametogenesis and gonadal steroid hormone synthesis. TSH acts on the thyroid gland, resulting in synthesis and secretion of thyroid hormones. All share a common subunit structure of α- and β-peptide chains which are non-covalently joined. The α-subunit (Figure 5.1) is common to all members of the this family whilst the β-subunits differ and confer hormonal specificity.

The subunits are biologically inactive in the free form; only the α–β heterodimer is able to bind to and stimulate the hormone receptors (reviewed by Pierce and Parsons, 1981). The peptide chains show close homology, the greatest being between the β-subunits of LH and CG (81%) (Figure 5.2).

hCG and LH bind to the same gonadal receptor, stimulating steroid synthesis and luteinization. The β-subunits are believed to have evolved from the common α-subunit gene (Fiddes and Talmadge, 1984). LH, FSH and TSH have been found in all mammals studied, and hormones with similar properties have been found in lower vertebrates, including teleost fish (reviewed by Pierce and Parsons, 1981). However, placenta-derived CG-like material is only found in two genera of mammals, primates and equids, having evolved separately within these two (Leigh and Stewart, 1990).

5.3.1 BIOSYNTHESIS

(a) Sources

Chorionic gonadotrophin is secreted by the syncytiotrophoblast cells of the placenta and is present in the blood and urine of pregnant women. First-trimester placental extracts have been shown to contain α- and β-

subunits of hCG, immature forms of these subunits and hCG itself (Tominaga *et al.*, 1989). There are other production sites which include trophoblastic or ectopic neoplasms. With the introduction of highly sensitive immunoassays, hCG or hCG-like material has also been detected in very small amounts in previously unsuspected conditions and tissues. Hartree *et al.* (1983) extracted material similar to hCG from the urine of some normal men and women and from the pituitary. They found quantities ranging from 0.5 to 1.1 μg per gland, which is some 25- to 50-fold less than the content of LH, FSH or TSH. The concentration in acetone-dried pituitaries ranges from 0.4 to 26 ng/mg which is close to that reported for full-term placental powder. The most active fraction was about 5% as potent as hCG itself; this was detected using antisera to the C-terminal residues 123–145 and 116–145, which react to hCG but not to LH. Minute amounts of hCG or the β-subunit have also been found in many normal tissues (Braunstein *et al.*, 1979; Yoshimoto *et al.*, 1979), in pituitaries (Matsuura *et al.*, 1980), especially of post-menopausal females (Armstrong *et al.*, 1984; Huang *et al.*, 1984), and in the urine of non-pregnant subjects (H.C. Chen *et al.*, 1978; D.M. Robertson *et al.*, 1978).

Yoshimoto *et al.* (1979) demonstrated the presence of hCG-like material in all human tissues they tested by a radioreceptor and a β-hCG radioimmunoassay. However, this material did not bind to concanavalin A as did placental hCG, and it was concluded that the material had the protein core of hCG but not the carbohydrate components. It would therefore have little or no biological activity *in vivo*. It seems that the trophoblastic cell is not unique in its ability to synthesize hCG sub-

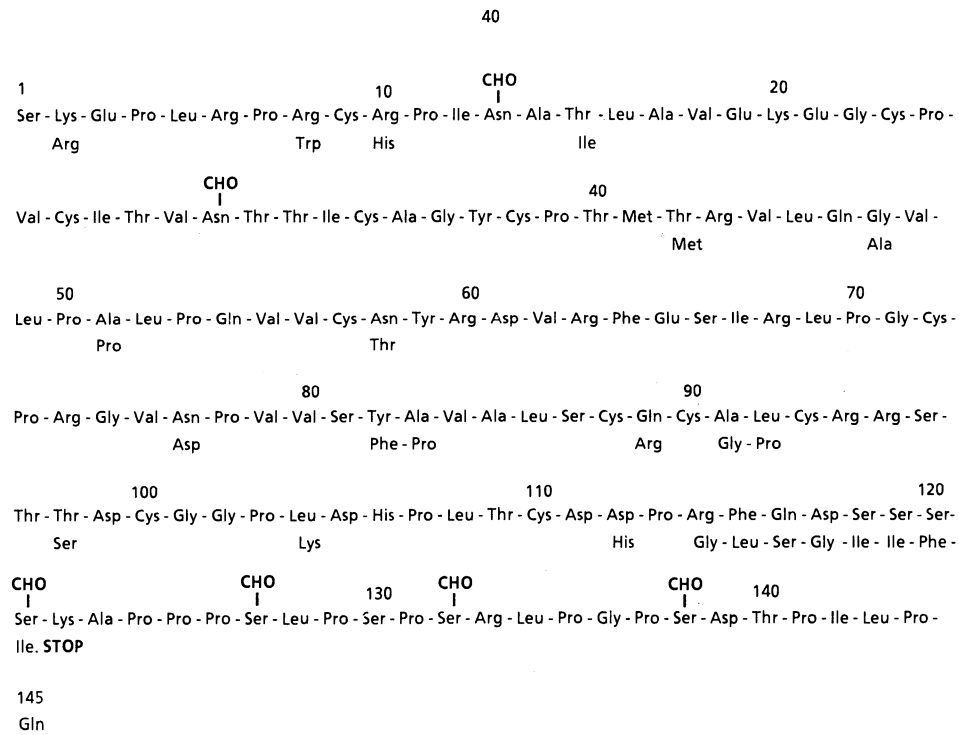

Figure 5.2 Amino acid sequence of the β-subunit of human chorionic gonadotrophin with β-subunit of luteinizing hormone residue differences shown below. CHO indicates positions of glycosylation. (Adapted from Hussa, 1987.)

units but is able to glycosylate and combine these cellular proteins into intact hCG. There is ample evidence for the presence of hCG (and hPL) in seminal plasma (Asch *et al.*, 1977; Ranta *et al.*, 1981; Bischof *et al.*, 1983; J.N. Lee *et al.*, 1983; Salem *et al.*, 1984a) but the levels do not relate to sperm numbers (J.N. Lee *et al.*, 1983). Chan *et al.* (1986) found detectable amounts of β-hCG in almost half the samples of seminal plasma studied. The concentrations were similar in samples from subjects with normal sperm counts and in those with oligospermia and with azoospermia. There were also no differences related to motility, morphology or fertilizing capacity and the physiological role of this β-hCG is at present uncertain. Placental proteins have also been found in ovarian follicular fluid (Westergaard *et al.*, 1985c).

(b) Synthesis of hCG

The common α-subunit is encoded by a single-copy gene on chromosome 6, and the β-subunits of FSH and TSH are encoded by single-copy genes on chromosomes 11 and 1 respectively. The β-subunit genes for LH and hCG are unique in that they form a cluster of seven genes/pseudogenes on the long arm of chromosome 19 (Boorstein *et al.*, 1982; Naylor *et al.*, 1983; Graham *et al.*, 1987). Owing to the close structural and sequence homology of these genes, all are believed to have evolved from a common ancestral α-subunit-like sequence (Fiddes and Goodman, 1980; Fiddes and Talmadge, 1984). This was thought to have been followed by further alterations in the upstream and downstream regulatory elements allowing for the selective expression of these individual peptides. The mechanism of increased gene transcription in pituitary and placental tissues, which specifically express these hormones, is mediated via the cAMP secondary messenger pathway. Though specific releasing hormones have been identified for the pituitary hormones, no known releasing factor has been identified for hCG. However, gonadotrophin-releasing hormone-like material has been identified in placental tissue (J.N. Lee *et al.*, 1981; Kim *et al.*, 1987), but it is not clear whether this is part of a specific control mechanism *in vivo*.

(c) The β-hCG–LH gene cluster

The common α-subunit gene was cloned from a placental mRNA library by Fiddes and Goodman (1979). Shortly afterwards they isolated the cDNA for the β-subunit of hCG (Fiddes and Goodman, 1980). Probing of genomic DNA with the cDNA clones soon established that only one α-subunit gene coded for the common α-subunit peptide of the glycoprotein hormones (Fiddes and Goodman, 1979; Boothby *et al.*, 1981). Analysis of human genomic libraries by Boorstein *et al.* (1982) and Policastro *et al.* (1983) revealed that multiple copy genes hybridized with the β-hCG cDNA. Boorstein *et al.* (1982) estimated that eight copies of β-hCG sequences were present in the human genome. The clone which was identified as 'gene 4' was subsequently shown to be β-LH. β-LH flanked the remaining genes which were arranged in tandem and inverted pairs on chromosome 19 (Julier *et al.*, 1984). This cluster had arisen from β-LH by base substitution in the TAA termination codon, leading to readthrough into the 3′ untranslated region, giving rise to the unique 30 amino acid carboxy-terminal extension (Fiddes and Goodman, 1980; Fiddes and Talmadge, 1984). Independent restriction and recombinant clone analysis by Policastro *et al.* (1986) identified only six β-hCG genes. The structural arrangements of the cluster and position mapping of the six β-hCG genes and β-LH were established by Graham *et al.* in 1987. This was subsequently confirmed by Jameson and Lindell (1988) (Figure 5.3).

The structural and functional organization of the β-hCG–LH gene cluster is of great interest because the presumed gene duplication from which it arose appears to have been followed by functionally significant

divergences within the coding and regulatory sequences (Talmadge *et al.*, 1984a).

(d) Equivalent materials in other species

High levels of chorionic gonadotrophin-like hormones have only been identified in pregnant primates and the pregnant horse. Placental expression of similar gonadotrophins has not been shown for other mammals (Pierce and Parsons, 1981). Using the known nucleic acid homology, β-LH probes have been used in an attempt to identify an equivalent β-CG gene in rats and cattle. Only a single β-LH gene could be detected in these species (Tepper and Roberts, 1984; Virgin *et al.*, 1985). Evidence for placental hormones in primates has been reviewed by Spies and Chappel (1984). There appear to be similarities in the structure of the CG from gorillas and chimpanzees to that from humans, but macaque CG seems to have different antigenic determinants. Tullner and Hertz (1966) recognized CG-like activity in the rhesus

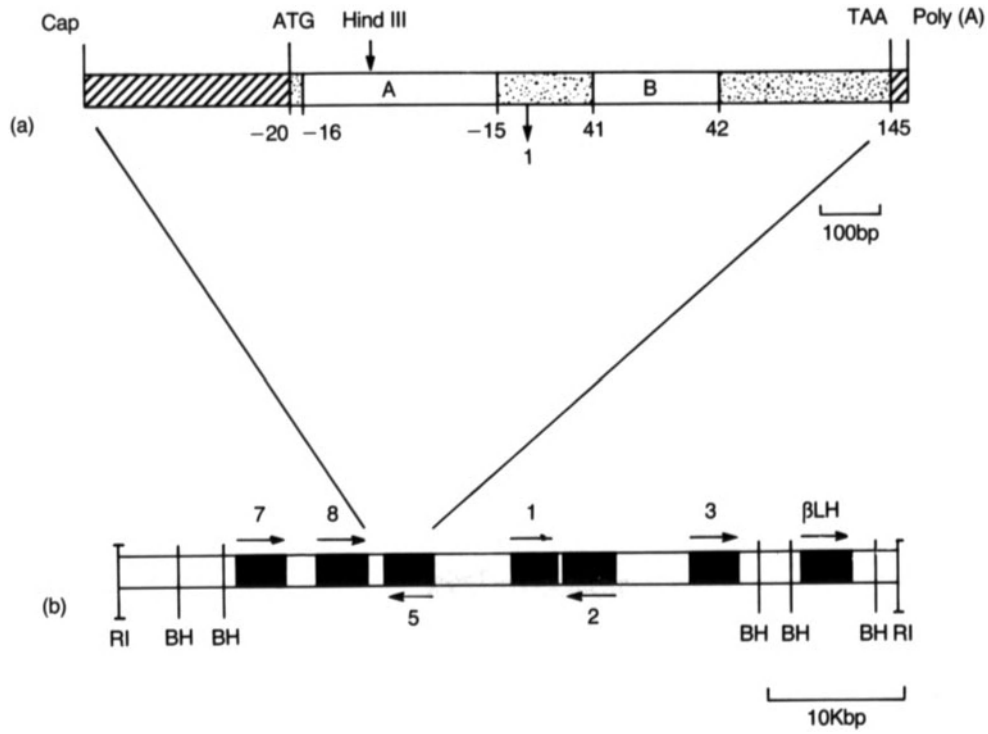

Figure 5.3 Composite structure of the β-subunit of the human chorionic gonadotrophin (β-hCG) gene and the β-hCG–hLH gene cluster. (Adapted from Boorstein *et al.*, 1982, and Graham *et al.*, 1987.) (A) The β-hCG gene/pseudogene structure: stippled areas represent exons and cross-hatched areas 5′ and 3′ untranslated regions. Introns A and B are marked, as are the cap site (Cap), initiation codon (ATG), termination codon (TAA) and polyadenylation site [Poly(A)]. The location of a single HindIII restriction site is also shown. Numbers refer to amino acids, negative numbers being signal peptides. (B) The β-hCG–hLH gene cluster: solid areas represent genes and pseudogenes. The position of the β-LH gene is marked. Numbers refer to β-hCG gene/pseudogenes which are labelled in the order of their isolation and initial sequence mapping (eight related sequences; Boorstein *et al.*, 1982). β-hCG gene 6 is an artefact of gene library construction and is therefore not mapped (gene 4 being β-LH Graham *et al.*, 1987). Arrows represent gene direction 5′–3′. The positions of *Eco*RI (RI) and *Bam*HI (BH) restriction sites are marked.

monkey from about the 35th day of pregnancy. Hobson and Wide (1972) extracted hCG from human placentas and monkey CG (mCG) from placentae of rhesus monkeys (*Macaca mulatta*) and marmosets (*Callithrix jacchus*). The mouse uterine weight test was used to measure the biological activities of hCG and mCG and both were neutralized by the addition of antisera raised to the hCG. There was no divergence from parallelism in the bioassays of the two preparations, but the radioimmunoassay to bioassay potency ratio was much higher than that of mCG.

Pregnancy gonadotrophins in equids have been intensively studied (Allen, 1979; Christakos and Bahl, 1979; Moore and Ward, 1980; Combarnous, 1981; Bousfield *et al.*, 1985). Pregnant mare serum gonadotrophin (PMSG) was first recognized in the 1930s (H.H. Cole and Hart, 1930; Zondek, 1930). It is produced in large quantities between the 40th and 150th days of gestation from the structures known as 'endometrial cups'. It is a glycoprotein with α- and β-subunits and, like hCG, has a C-terminal extension, but it differs from hCG by having FSH-like activity as well as LH.

A CG from the donkey was purified and partially characterized from serum (Aggarwal *et al.*, 1980). This proved to be a glycoprotein with a similar molecular size to the mare gonadotrophin but with slightly less (31% compared with 45%) carbohydrate. In bioassays it was shown to be predominantly LH-like.

The additional carboxy-terminal peptides of the horse and donkey β-CG have very little (if any) amino acid homology with those of the human and the baboon. The frameshift that gave rise to the human and baboon CG carboxy-terminal extension was the result of a single nucleotide deletion at codon 114 of the β-LH gene. In the absence of a published sequence for an equid β-LH gene, the horse and donkey β-CG sequences have been compared with the β-LH sequence of the cow. This has shown that the frameshift responsible for the carboxy-terminal extension is due to a nine nucleotide deletion from codon 112. In addition, there is a loss of amino sequence homology for horse and donkey β-CGs from codon 127. This is due to the insertion of nine additional nucleotides at this point in the donkey β-CG sequence, suggesting independent evolution of β-CG sequences for primates and equids, and indications that further mutations have occurred since the evolutionary divergence of the horse and donkey (Leigh and Stewart, 1990). However, the amino acid sequence of horse β-LH is identical to that of horse placentally derived β-CG (Bousfield *et al.*, 1987). This suggested that in equids the stop codon frameshift which gave rise to the carboxy-terminal extension occurred in the horse β-LH gene. This has now been confirmed by F. Stewart and Maher (1991) and Sherman *et al.* (1992), who have shown that the horse has only a single copy of the β-LH/CG gene. Initial studies also suggest that the same promoter and enhancer elements are responsible for placental and pituitary expression of this equine gonadotropic gene; this is not the case for human expression of CG and LH (Knoll, 1992; Sherman *et al.*, 1992). The variable frameshift and gene duplication events giving rise to β-hCG and lengthened equine β-LH/CG sequences suggest a degree of genetic instability in the ancestral β-LH gene. It would therefore be of great interest to map the evolution of the human β-CG-LH gene cluster through primates of the New and Old Worlds.

(e) β-hCG gene expression

Using transfection into simian virus 40 (SV40)-transformed Chinese hamster ovary (CHO) cells and sequence analysis, Talmadge *et al.* (1984b) found that only β-hCG genes 3, 5 and possibly 1 were transcribed in *in vitro* studies. However, the influence of the promoter sequence SV40 virus could not be excluded. Transfection into non-transformed

mouse Y1 cells (Otani *et al.*, 1988) showed that genes 3, 5 and 8 were transcriptionally active. Assuming that all the genes are regulated by similar promoter and enhancer sequences 5′ of the initiation codon, only genes 3, 5 and 8 are functional. Analysis of mRNA extracted from placental and choriocarcinoma cells by Jameson *et al.* (1987) demonstrated that transcription of β-hCG gene 5 is much the most active (20:1 relative to gene 3). This is in agreement with Otani *et al.* (1988), who found that gene 5 was the most actively expressed, followed by gene 3, then gene 8. A recent study has addressed which of the gene/pseudogenes are silent *in vivo*. Bo and Boime (1992) examined β-hCG mRNA from placenta and from the choriocarcinoma cell line JAR. Though message was detectable from genes 5, 3 and 8 in the expected order of magnitude, message was also detectable from genes 7 and 1 or 2. Furthermore, the message arising from either gene 1 or 2 was shorter than those from the others because of alternative splicing. This indicates that at least five of the six genes are transcriptionally active but that one may undergo alternative splicing. Multiple promoter and enhancer regions are present within 200 bp 5′ of gene 5 of the β-hCG gene cluster (Fenstermaker *et al.*, 1989). However, no known promoter sequences such as the CAAT and TATAA boxes can be found upstream of the cap site of these genes. Expression of β-hCG and α-subunit mRNA by trophoblast and choriocarcinoma cells is responsive to adenylate cyclase/cAMP. Alpha-subunit mRNA expression is 10 times faster than that of β-hCG. This suggests that the activation pathways of regulatory DNA-binding nuclear proteins acting on the cAMP response elements (CREs) adjacent to the α-subunit are not the same as those of the β-hCG genes (Milstead *et al.*, 1987; Anderson *et al.*, 1988; Fenstermaker *et al.*, 1989). All of the glycoprotein hormone genes contain 5′ CRE enhancers which act in conjunction with specific pituitary or placental promoters (Chin, 1988; Jameson and Lindell, 1988; Fenstermaker *et al.*, 1989; Balfour *et al.*, 1990). Expression of the common α-subunit must depend on multiple promoter regions responsive to specific DNA-binding factors occurring in pituitary gonadotrophs and thyrotrophs and in the placental trophoblast (Balfour *et al.*, 1990). Placental expression of the α-subunit only occurs in primates and horses. Unlike the α-subunit genes of the rat (Burnside *et al.*, 1988) and the mouse (D.F. Gordon *et al.*, 1988), which have only one CRE, multiple copies of CRE sequences can be found 5′ of the cap site of the α-subunit gene of primates and horses (Chin and Gharib, 1986). This suggests that a newly evolved promoter element specific to the trophoblast interacts with copies of the CRE enhancer distinct from those involved in pituitary expression of this gene (Knoll, 1992). Down-regulation of the α-subunit in the pituitary is achieved by oestrogen and/ or thyroid hormone acting via nuclear receptors, such as c-ErbA, and specific regulatory elements. For example, down-regulating sequences such as the thyroid response element, which regulates the expression of β-TSH, are also present in the α-subunit gene (Chin, 1988). No such negative-feedback control mechanism has been identified for the β-hCG genes.

(f) Biosynthesis, glycosylation and combination of hCG subunits

Synthesis of intact hCG, like all the glycoprotein hormones, requires the simultaneous activation of both the α- and β-genes. The rate-limiting component of the production of intact hCG and the other glycoprotein hormones is thought to be the rate of the synthesis of the β-subunit. This is supported by evidence that the α-subunit is produced in excess by most glycoprotein hormone-secreting cells. The studies of Milstead *et al.* (1987), which showed much faster transcription of the α-subunit gene in response to

cAMP, would also support this assumption. In pregnancy, maternal serum contains an excess of free β-hCG in the first 3 or 4 weeks post implantation; this is rapidly superseded by large quantities of intact hCG, with very little free β-subunit but a steady increase of free α-subunit (L.A. Cole *et al.*, 1984a; Norman *et al.*, 1985a; Alfthan *et al.*, 1988; L.A. Cole *et al.*, 1990). Kaetzel and Nilson (1988) have suggested that β-LH transcription is not the rate-limiting step in intact LH synthesis. When α-subunit and β-LH genes were co-transfected into CHO cells, selective amplification of the α-subunit gene led to an increase in expression of intact LH. Nevertheless, the pulse-chase experiments of Ruddon *et al.* (1987, 1988) have indicated that the newly synthesized β-LH and β-hCG require time to achieve a conformation appropriate for combination with the α-subunit, thus suggesting that β-subunit synthesis is the rate-limiting determinant. Furthermore, direct comparison between the rates of synthesis of LH and hCG is not possible.

The transfection studies of Corless *et al.* (1987) have shown that free β-hCG, once synthesized, is rapidly secreted. β-LH, by contrast, requires association with the α-subunit before it can be secreted. Until this is achieved, 90% of newly synthesized free β-LH is stored in the cell. α–β association for LH was found to be slow and of poor efficiency. The transfected β-hCG gene product rapidly associated with free α-subunit with high efficiency. In agreement with these studies, Handwerger *et al.* (1987) found that intact hCG and free β-hCG are not stored in trophoblastic secretory granules. By contrast, the free α-subunit can easily be detected in pituitary gonadotroph and placental trophoblast cells. The pulse-chase studies on the JAR choriocarcinoma cell line indicate that precursors of α- and β-subunits undergo changes which allow subunit association (Ruddon *et al.*, 1988). Glycosylation occurs after these crucial conformational changes (Ruddon *et al.*, 1987, 1988). This order of events is supported by the isolation of an incompletely glycosylated form of intact hCG from human placental tissue (H. Wang *et al.*, 1988). No *O*-linked sugars could be detected, only shortened *N*-linked moieties. It is therefore possible that *N*-linked glycosylation begins at the same time as the synthesis of precursors, while *O*-linked sugars are added at the time of subunit association. It should be noted that in these studies only 50% of the β-hCG synthesized by the JAR cell line combined with the α-subunit despite an excess of free α-subunit. This may reflect defects in the processing pathway of this and other hCG-producing carcinomas.

5.3.2 METABOLISM

Only 22% of serum hCG is excreted intact into the urine (Wehmann and Nisula, 1981). Surprisingly, dissociated free α- and β-subunits of hCG are not found in the urine of subjects infused with purified intact hCG. This is not true of pregnancy urine, in which these free subunits are readily identified (Nisula *et al.*, 1989). Clearly the majority of intact hCG is processed by mechanisms other than direct urinary clearance (Sowers *et al.*, 1979). The free subunits of hCG have much faster clearance rates than intact hCG: the mean clearance rate (MCR) of intact hCG is 1.9 ml/min m^2; free α-subunit 50 ml/min m^2; free β-subunit 19 ml/min m^2 (Sowers *et al.*, 1979; Wehmann and Nisula, 1980a; Wehmann *et al.*, 1989). Less than 1% of these free subunits are excreted unaltered into the urine (Wehmann and Nisula, 1980a).

Animal studies have shown that hCG is taken up by the liver, kidney and ovaries. The kidneys are the most important, accounting for 70–80% (Markkanen *et al.*, 1979; K.L. Campbell *et al.*, 1981; Nisula *et al.*, 1989). Hepatic absorption is predominantly via the Kupffer cells of the liver sinusoids and ovarian by the theca/luteal cells. Renal absorption is by cells of the proximal tubules

(Markkanen *et al.*, 1979; K.L. Campbell *et al.*, 1981). Unlike the liver and ovary, virtually no intact hormone can be recovered from kidney tissue homogenates. Kidney homogenate studies have also shown that α-subunit is processed much more rapidly than the β-subunit, which is also retained for longer (Nisula *et al.*, 1989). Whilst desialylation increases hCG MCR by up to 200 times, the quantity of desialylated intact hCG excreted directly into the urine falls to less than 2.5% of that infused (Rosa *et al.*, 1984; Nisula *et al.*, 1989). However, desialylation, and hence exposure of terminal galactose sugars, does not affect liver uptake, nor does blockade of hepatic galactose receptors (Nisula *et al.*, 1989).

A major immunoreactive form of β-hCG found in pregnancy urine is a low molecular weight species clearly distinguishable on Sephadex G-100 exclusion chromatography from intact and free β-subunit of hCG (Kato and Braunstein, 1988). Originally reported in pregnancy urine by Franchimont *et al.* (1972), similar material was also found in the urine of patients with hCG-expressing tumours (Vaitukaitis, 1973). This degradation product of the β-subunit of hCG has been given various names; the commonest designation, 'β-core fragment', is so named because it retains immunological determinants common to the 'core' structure of hCG but not those of the carboxy-terminal peptide (Akar *et al.*, 1988; Wehmann *et al.*, 1987). The β-core fragment has only recently been fully characterized. It consists of two polypeptide chains resulting from tryptic degradation of the β-subunit of hCG. Amino acid residues 6–40 and 55–92 of this subunit, joined by disulphide bridges, constitute the β-core fragment (Birken *et al.*, 1988). It therefore lacks the unique carboxy-terminal peptide and LH/hCG receptor-binding regions. Because it retains conformational immunological determinants common to intact and free β-hCG, it is recognized by many polyclonal antisera raised against (β)-hCG (Iles *et al.*, 1990). β-core is essentially undetectable (< 0.2 ng/ml) in the sera of pregnant women or volunteers and animals infused with exogenous intact hCG and β-hCG. Though detectable in the urine, its appearance in experimental animals and volunteers is delayed for up to 24 h, despite detection of urinary intact hCG very shortly after administration. Furthermore, analysis of rat tissue homogenates following absorption of exogenous hCG reveals immunoreactive β-hCG-like material of the same molecular size as β-core on Sephadex G-100 chromatography (Nisula *et al.*, 1989). Purified β-core infused into volunteers is cleared 100 times faster than intact hCG (MCR 192.0 ml/min m^2). However, further degradation is likely since only 9% of infused material is recovered in the urine (Wehmann *et al.*, 1989). The measurable urinary clearance rate of intact hCG, subunits and core is much lower than the circulatory clearance rates. It is therefore evident that uptake and processing of hCG by body organs, most probably by cells of the renal proximal tubules, is the principal route for hCG metabolism. β-core is therefore likely to be one of these metabolic products and the predominant form of β-hCG immunoreactivity in urine (Wehmann and Nisula, 1980b; Nisula *et al.*, 1989; Wehmann *et al.*, 1989, 1990).

Despite its apparent absence in sera, several workers have suggested that β-core is a distinct product of the placenta and neoplasms (L.A. Cole and Birken, 1988; Kardana *et al.*, 1988). Recently, it has been suggested that the urinary β-core fragment of human chorionic gonadotrophin (hCG) may be a marker in women with non-trophoblastic gynaecological malignancy (L.A. Cole *et al.*, 1988; Y. Wang *et al.*, 1988; Nam *et al.*, 1989; C.L. Lee *et al.*, 1991). This is largely based on the observation that β-core can often be the sole measurable hCG-related tumour marker in some cancer patients (Papapetrou and Nicopoulou, 1986).

More recent studies have shown that immunoreactive β-core-like material is present in urine of pre- and post-menopausal women

with no malignant disease. The levels are five times higher in the post- than the pre-menopausal woman (C.L. Lee *et al.*, 1991). Immunochemical characterization of this post-menopausal material revealed that it was of the same molecular size as the β-core of hCG but considerably more immunoreactive in LH assays than authentic β-core of pregnancy. Significant quantities of intact hCG are not found in the blood or urine of post-menopausal women; however, LH levels are elevated. Theoretically, a breakdown product similar to hCG β-core should result from the metabolism of LH in the kidney. The amino acid sequence of a putative LH β-core would be virtually identical to that of hCG-β-core (Iles *et al.*, 1992).

The process by which intact hCG is metabolized would appear to begin in the blood with the cleavage ('nicking') of internal bonds at residues 43–49 of the β-subunit whilst it is still associated with the α-subunit (L.A. Cole *et al.*, 1993). This is the centre of a major binding loop (residues 38–57) common to both LH and hCG (Ryan *et al.*, 1987). This region is also missing in the β-core molecule of hCG. Identical 'nicking' was described in preparations of LH 20 years earlier (Shome and Parlow, 1973). Thus, both nicked intact hCG and nicked LH have been identified, suggesting a common degradation pathway. The existence of a β-LH core implies the existence of β-TSH core and β-FSH core molecules (Iles *et al.*, 1992).

5.3.3 CHEMISTRY

(a) Methods of extraction

The earliest methods depended on precipitation from urine with acetone or ethanol, but adsorption to benzoic acid is more economical (Katzman and Doisy, 1934). The benzoic acid precipitate can be dissolved in acetone or ethanol, which simultaneously precipitates the adsorbed protein. Adsorption of the hormone from acidified urine to Permutit (Katzman *et al.*, 1943) or to kaolin with subsequent elution in alkali and precipitation from ethanol has also been widely used. Methods are also available for extracting the hormone from placental tissue, trophoblastic tumours or from male choriocarcinomas (Bates *et al.*, 1968; Ashitaka *et al.*, 1972; Pala *et al.*, 1973).

(b) Methods of purification

In early work on hCG, the studies of Got and Bourrillon (1960) are notable because of the thoroughness and care with which they were carried out. Benzoic acid extracts were fractionated on Permutit using a mixture of ammonium acetate and ethanol (60:40, v/v). A potency of 13 000 IU/mg was obtained and some of the chemical characteristics were listed, including the fact that the product was a glycoprotein with approximately 30% by weight of carbohydrate.

Purification procedures reported by later workers have been complicated by the considerable heterogeneity found in the hCG molecule, primarily because of variations in carbohydrate structure. van Hell *et al.* (1968) and Bell *et al.* (1969) used CM-Sephadex or cellulose, gel filtration on G-100 and isoelectric focusing. In the pH range 3–5, several species of molecule were recognized with biological activities ranging from 1700 i.u. to 18 000 iu/mg. The potencies correlated well with the sialic acid contents which varied from 2.9 to 10.7%. Goverde *et al.* (1968) found that a loss of 0.3% sialic acid lowered the biological activity from 18 000 to 12 000. The protein portions of these molecules had relatively constant amino acid composition and were immunologically identical.

Preparations of hCG purified from commercial-grade material by Bell *et al.* (1969) and Canfield *et al.* (1971) have been widely used. Conventional techniques including chromatography on ion exchange and gel filtration yielded material with biological activity consistently between 13 000 and 15 000 iu/mg.

Clearly the definition of purity is not straightforward because of variations in the sialic acid content. Canfield *et al.* (1971) suggested that one criterion for purity should be an analysis of the protein content of the hormone, with 12 proline residues per 100 residues, as was obtained in their own purified material.

(c) Properties

The molecular weight of hCG (38 000) is higher than that of the other glycoprotein hormones because of the extension at the C-terminus of the β-subunit (F.J. Morgan *et al.*, 1975). The isoelectric point, 2.9–3.5, is lower than that of the pituitary gonadotrophins on account of the high sialic acid content, which has been estimated to be about 8.5% (Bahl, 1969; Mori, 1970). The Stokes' radius is 30 Å and the hydrodynamic radius about 25 Å.

(d) Structure in relation to function

The α- and β-subunits of hCG can be reversibly dissociated, as can those of the other glycoprotein hormones (Gray, 1988). The amino acid sequence of the α-subunit (Ryan *et al.*, 1987) is identical to that of the α-subunits of the glycoprotein hormones of the pituitary, but in almost all studies so far some heterogeneity has been reported at the N-terminus. Keutmann *et al.* (1978) found that the full 92 residues (Figure 5.2) made up 60% of the α-subunit, while 30% lacked the last three residues and 10% the last two residues at the N-terminus. However, these forms all recombine with β-subunits to give biologically active molecules so that heterogeneity is apparently of no consequence for subunit interactions or for biological activity.

The first 115 amino acids of β-hCG share 82% homology with β-hLH. Furthermore, there is more than 40% amino acid homology among all the β-subunits of this family. The most conserved regions are around the 12 cysteine residues (Pierce and Parsons, 1981). This suggests similarity in the folding patterns of the β-subunits, a concept which is further supported by the fact that the common α-subunit associates with all β-subunits. In addition, structural domains exposed by common disulphide bonds may be involved in subunit–receptor as well as subunit–subunit interactions; there is significant binding activity of hCG with the LH and the TSH receptors (Ryan *et al.*, 1987). Thus, there are many common secondary and tertiary domains which are probably accounted for by the disulphide linkages.

Disulphide bridges

Full analysis is available for the disulphide bonds of the β-subunits of ovine LH (D. Chang *et al.*, 1975; Tsunasawara *et al.*, 1977), bovine LH and TSH (Reeve *et al.*, 1975), human CG (Mise and Bahl, 1981) and human FSH (Fujiki *et al.*, 1980) (see Table 5.1a).

Despite the conserved position of the half cysteines there is only agreement on the pairing of three of the six bridges: Cys-23–Cys-72, Cys-26–Cys 110 and Cys-9–Cys-100. Detailed analysis is difficult because the disulphide bonds are clustered in regions where few intervening peptide bonds are readily susceptible to hydrolysis. Based on partial reduction and S-^{14}C-carboxy-methylation, Mise and Bahl (1981) assigned the remaining three pairs as Cys-9–Cys-90, Cys-34–Cys-88 and Cys-38–Cys-57. Willey and Leidenberger (1989) constructed a three dimensional molecular model based on published findings, agonist receptor binding, epitope mapping and computer modelling by hydrophobic region/comparative sequence analysis. This assigned the remaining hCG disulphide bridges as Cys-23–Cys88, Cys-34–Cys-57 and Cys-38–Cys-72. Mise and Bahl (1981) postulated that a large receptor-binding loop was stabilized by cysteine residues 38 and 57. Synthesis of this region yielded an agonist with moderate binding activity in a competi-

Table 5.1 Assignment of disulphide bond partners for ovine (o) and bovine (b) LH and human chorionic gonadotrophin (hCG) subunits

α-subunit

oLH	*oLH*	*bLH*	*hCG*	*hCG*
Chang et al.	*Tsunasawara* et al.	*Reeve* et al.	*Mise and Bahl*	*Willey and Leidenberger*
(1975)	*(1977)*	*(1975)*	*(1981)*	*(1989)*
9–38	9–90		9–90	9–90
23–72	23–72		23–72	23–88
26–110	26–110		26–110	26–110
34–90	34–88		34–88	34–57
57–88	38–57		38–57	38–72
93–100	93–100	93–100	93–100	93–100

ß-subunit

oLH	*bLH*	*hCG*	*hCG*
Chang et al.	*Cornell and Pierce*	*Mise and Bahl*	*Willey and Leidenberger*
(1973)	*(1974)*	*(1980)*	*(1989)*
11–64	11–35	11–35	11–35
14–36	14–36	14–36	14–88
32–88	32–64	32–64	32–64
35–63	63–91	63–91	63–91
36–91	86–88	96–88	86–36

tive hCG/LH receptor assay (Keutmann *et al.*, 1987; Ryan *et al.*, 1988).

The pairing of Cys-38 with Cys-72 by Willey and Leidenberger (1989) is largely based on a comparison of the sequence and tertiary structure of the intact hCG α–β combined receptor-binding domain with those of the substrate-binding cleft of bovine chymotrypsin. Antigenic mapping studies have shown that some epitopes present in the free β-subunit, particularly those specific for the β Cys-38–Cys-57 loop, are lost upon association with the β-subunit. These epitopes are not masked by the β-subunit; instead, association of the subunits leads to a conformational change, and new epitopes specific for this region arise (R.K. Campbell *et al.*, 1988). Furthermore, intracellular forms of β-hCG have been detected which cannot associate with the free α-subunit. This is possibly due to aberration in the intramolecular disulphide bridges (Ruddon *et al.*, 1988).

The importance of the remaining disulphide bonds in subunit–subunit, secretion and receptor interactions has not been fully established (Ryan *et al.*, 1988). The disulphide pair β-Cys-93–Cys-100 is most accessible to reduction. Since it is part of a receptor-binding region, reduction is associated with loss of biological activity (Pierce and Parsons, 1981; Ryan *et al.*, 1988; Willey and Leidenberger, 1989). Based on the disulphide pairing of Mise and Bahl (1981), a series of site-directed mutagenic constructs replacing the specific cysteine codons for serine or alanine in the β-hCG gene were transfected into CHO cells by Suganuma *et al.* (1989). The normal α-subunit gene was co-transfected

and the proposed disulphide bridge partners of the β-subunit were disrupted by mutating one or both cysteines. The effects of such changes on assembly and secretion of intact hCG were studied by comparing *in vitro* secretion of hCG by CHO cells containing transfected wild-type genes with those containing the mutants. Surprisingly, the findings were inconsistent for individual and paired cysteine mutations. The secretion of intact hCG was greatly reduced with single mutations at Cys-26, -110, -72 and -90, but was reduced too a much lesser extent if the proposed partner was jointly mutated. All other mutations resulted in non-formation of the heterodimer of intact hCG. The free β-subunit of hCG is rapidly secreted once formed. Comparison of the secretion rates of mutant and wild-type genes showed that if intact hCG was not formed the rate of β-hCG secretion was increased. Thus, alteration of cysteine residues impedes and, in most cases, prevents α–β subunit association. However, the stability of the free β-subunit is unaffected.

The pairing of the five disulphide bonds of the α-subunit is also disputed (see Table 5.1b) (Ryan *et al.*, 1987). For example, Willey and Leidenberger (1989) have inverted two of the pairings assigned by the widely accepted reduction/alkylation studies of Cornell and Pierce (1974) and Mise and Bahl (1980) (i.e. Cys-14–Cys-88, Cys-36–Cys-86 instead of Cys-14–Cys-36, Cys-86–Cys-88). In addition, a form of α-subunit which does not combine with the β-subunit has been identified (Beebe *et al.*, 1989). Finally, some of the antigenic conformational changes that occur when the α- and β-subunits combine may be a consequence of intradisulphide bridge partner exchange on both molecules.

Helices and β-pleated sheets

No successful X-ray crystallographic examination of hCG has been achieved. However, estimates of the relative proportions of secondary structural components have been obtained from optical dichroism studies (reviewed by Pierce and Parsons, 1981). For the glycoprotein hormones as a whole, α-helix content is low (5–8%) and the content of β-pleated structures is relatively high (25–40%). Computer modelling based on primary sequence data shows that all β-subunits exhibit homologous regions of extended β-pleated sheeting. For β-hCG the estimates are: 1% α-helices, 71% β-pleated sheeting with 27% β-turns, and 11% random coils. With the two subunits combined the proportion of β-pleated sheeting is reduced to 61% (Pierce and Parsons, 1981). The numerous disulphide bridges indicate a high degree of β-pleated sheeting and the close sequence pairing of cysteine partners is consistent with the high content of β-turns (i.e. α-Cys-86–Cys-88, β-Cys-93–Cys-100).

Glycosylation – heterogeneity and function

The glycoprotein hormones are characterized by large sugar moieties linked to the asparagine (Asn) residues of both the α- and β-chains. The common α-subunit has two *N*-glycosidic-linked complex branched dodecasaccharide moieties on Asn-52 and Asn-78. All the β-subunits have two similar *N*-glycosidic moieties on Asn-13 and Asn-30. In addition, β-hCG has four serine *O*-glycosidic-linked, tetrasaccharide, mucin-type moieties on residues 121, 127, 132 and 138 of the unique carboxy-terminal extension. The composition and biantennae structural arrangement of the sugar moieties, *N*- (Figure 5.4) and *O*- (Figure 5.5) glycosidic, elucidated for pregnancy hCG by Kessler *et al.*, (1979a,b) is still widely accepted.

Microheterogeneity has frequently been found in the structure of these carbohydrate moieties (Endo *et al.*, 1979; Kennedy and Chaplin, 1976; Mizuochi and Kobata, 1980; Bahl and Wagh, 1986; Kobata 1988). hCG oligosaccharides contain *N*-acetylneuraminic acid (NANA, sialic acid) attached exclusively

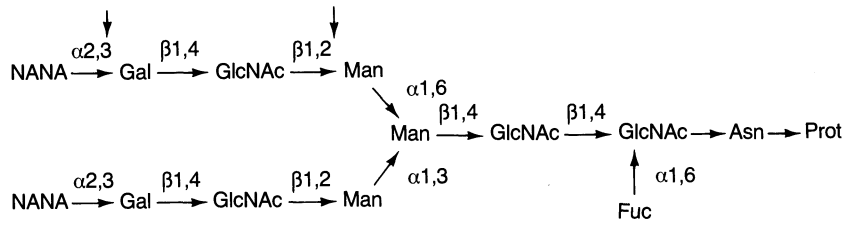

Figure 5.4 Carbohydrate structure of *N*-glycosidic moieties in α- and β-subunits of hCG (Bahl and Wagh, 1986; Ryan *et al.*, 1987). Some chains have been reported to terminate at the points indicated by the arrows. The fucose residue is present only in the β-subunit. NANA: *N*-acetylneuraminic acid (sialic acid); Gal: galactose; Man: mannose; Fuc: fucose; GlcNAc: *N*-acetylglucosamine.

by 2,3 linkage to galactose residues rather than the 2,6 linkage more commonly found in serum glycoproteins such as transferrin and immunoglobulins. The functional significance of this is unknown. Heterogeneity has been reported in the fucose residues attached in some chains to the inner *N*-acetylglucosamine (GlcNAc) residue. The asparagine complex on the α-subunit is not fucosylated. A monoantenna form of this asparagine complex has also been identified in normal pregnancy hCG. The Kessler classic form and the two variant forms described are distributed on the α- and β-subunits of pregnancy hCG in a set ratio (Kobata, 1988). Small amounts of triantennary forms have been described on the β-subunit of pregnancy hCG (Damm *et al.*, 1988). Similar heterogeneity has been described for the serine *O*-linked carbohydrates moieties by L.A. Cole (1987) who identified hexasaccharide, trisaccharide and disaccharide forms in addition to the tetrasaccharide.

Differences in the composition of the oligosaccharides in the α-subunit of hCG and the pituitary glycoprotein α-subunits have been recognized (Nilsson *et al.*, 1986), and although terminal sulphated carbohydrates exist in α-LH none have been found in α-hCG (Parsons and Pierce, 1980). This is explained by the different oligosaccharide-processing enzymes present in the tissues of origin: sulphation of LH in the pituitary is due to sulphotransferase activity not found in the placenta (Green *et al.*, 1986). Blithe and Nisula (1985) recognized differences between the oligosaccharide structures contained in the α-subunit of hCG obtained by dissociation of hCG itself and the forms of the free α-subunit which are recognized in urine of pregnant women. A major difference appeared to be in the higher sialic acid content of the free forms.

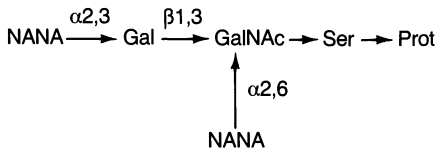

Figure 5.5 Carbohydrate structure of *O*-glycosidic moieties in β-hCG (Kessler *et al.*, 1979b). GalNAc: *N*-acetylgalactosamine.

A hyperglycosylated variant of free β-subunit has been isolated from pregnancy serum and hCG-secreting trophoblast cultures; this contains an additional mucin-type sugar residue *O*-linked to Thr-39 (L.A. Cole *et al.*, 1984b). First identified by Parsons *et al.* (1983) in bovine pituitary extracts, the additional glycosylation prevented subunit association with bovine β-LH (Parsons and Pierce, 1984). However, some studies have shown that the presence of this additional sugar moiety does not affect assembly of the α–β heterodimer (Peters *et al.*, 1989).

Heterogeneity has also been noted in the peripheral chains, some terminating in

NANA and others in galactose or mannose. Whether these differences are related to variations in the post-translational operations which occur during biosynthesis or to degradation during purification is not known.

In neoplastic expression of hCG, considerable variability in the glycosylation has been found (Yoshimoto *et al.*, 1979). The most striking cause of physicochemical heterogeneity of hCG is the variation in the proportion of terminal sialic acid residues present on these sugar moieties. This is responsible for the numerous acidic isoforms of hCG. Yazaki *et al.* (1980) and many others have demonstrated multiple isoforms of pregnancy hCG with pI values between 3.9 and 6.0. The hCG produced by gestational trophoblastic tumours shows increased glycosylation with triantennary branches of the asparagine–sugar moieties. Urinary hCG from patients with choriocarcinoma has a reduced number of terminal sialic acid residues, despite an increase in antennae susceptible to sialation (Nishimura *et al.*, 1981; Mizuochi *et al.*, 1983; Kobata, 1988). This is in contrast to the shift towards acidic isoforms of hCG (predominantly pI 3.5, 3.8 and 4.0) found in serum from choriocarcinoma patients (Yazaki *et al.*, 1980; Mann *et al.*, 1986; Kobata, 1988; L.A. Cole *et al.*, 1990).

In normal pregnancy, isoforms of pI 3.9, 4.0, 4.2 and 4.4 are the most prevalent, with a shift towards the more basic forms as pregnancy progresses (L.A. Cole *et al.*, 1990). This pI isoform shift may be reflected in the ratio of biological assay potency to immunoassay estimation (B/I); this ratio is greatest in early pregnancy (reviewed by Stockell Hartree, 1989). Two factors may explain this changing ratio. First, the presence of sialic acid decreases the rate of hCG clearance from the peripheral circulation because of reduced binding to hepatic lectins (Van Hall *et al.*, 1971). Second, though the affinity of hCG for the LH–hCG receptor is enhanced, the ability of the deglycosylated hCG molecules to trigger a cAMP response is diminished (H.C. Chen *et al.*, 1982; Sairam and Bhargavi, 1985; Keene *et al.*, 1989). These sugar-depleted molecules can act as an antagonist (H.C. Chen *et al.*, 1982; Ranta *et al.*, 1987).

Similar variation in glycosylation is seen with other glycoprotein hormones. Changes in glycosylation may represent control mechanisms for LH, FSH and hCG bioactivity. Changes in pI and B/I ratio are seen during the menstrual cycle and at different reproductive stages such as puberty and the menopause (reviewed by C.A. Wilson *et al.*, 1990).

The carboxy-terminal extension of hCG

β-hCG possesses a 30 amino acid carboxy-terminal extension, which arose following evolution from β-LH. It contains four sites of mucin-type glycosylation at serine residues 121, 127, 132 and 138. Many highly specific assays have been developed using antibodies directed against this region. Indeed, synthetic peptides corresponding to this region constitute the WHO contraceptive vaccine (Stevens, 1986). The role of this unique region in hCG function has only recently been established (Matzuk *et al.*, 1990). Using site-directed mutagenesis, a termination codon (TAA) was introduced at codon 115 of the hCG gene to produce a truncated peptide similar to β-LH. This construct was transfected together with the wild-type α-subunit gene into CHO cells. To address the role of the mucin-like sugar moieties of this region, wild-type α- and β-hCG genes were also co-transfected into the *O*-linked glycosylation-defective murine cell line IdID. The expressed mutant hormones were tested for receptor binding and gonadotrophic activity. *In vitro*, the truncated and *O*-linked glycosylation mutant forms had the same receptor-binding characteristics and stimulatory effects as the wild-type hCG. This finding is consistent with immunological studies which show that the carboxy-terminal region is a surface structure which is still exposed when

hCG binds to the LH–hCG receptor. Thus, this region contributes little to tertiary structure and receptor-binding function (Louvet *et al.*, 1974; Bidart *et al.*, 1987). The carboxy-terminal region has no effect on β-hCG gene expression or α–β subunit assembly (El-Deiry *et al.*, 1989, Matzuk *et al.*, 1990). The *in vivo* activities of the truncated form and glycosylation mutants were 3–4 times less than that of the wild type (Matzuk *et al.*, 1990). This finding is consistent with the glycosylation studies and suggests that the biological function of the glycosylated carboxy-terminal extension is to prolong the circulating half-life of intact hCG.

Theoretical tertiary and quaternary models

Although the linear sequences of all the glycoprotein hormone subunits have been known for over 15 years, little progress has been made in determining the three-dimensional structures. Heterogeneity in the carbohydrate moieties prevents the formation of crystal suitable for X-ray diffraction studies. Only two groups have described the formation of hCG crystals suitable for X-ray analysis after chemical or enzymatic reduction of the carbohydrate moieties with hydrogen fluoride or neuraminidase (Harris *et al.*, 1989; Lustbader *et al.*, 1989). Nevertheless, two tertiary models have been proposed: one by Moyle, Lustbar and Canfield (discussion section of Ryan *et al.*, 1987) is based on the disulphide bond assignment of Mise and Bahl (1981). The second, by Willey and Leidenberger (1989), is based on their assignments of the disulphide bonds. The main differences between the models are in the assignment of three pairs of disulphide bonds on the β-subunit and a further two on the α-subunit. Synthetic peptides have been made which emulate various segments of the α- and β-subunits of LH and hCG. These have been used to determine which peptides bind to the LH–hCG receptor, stimulate testosterone production in the Leydig cell assays and block intact hCG or LH activity. Considerably more peptide (typically 10^9) was required to compete with or match intact hCG (Ryan *et al.*, 1987). Inhibitory binding constants, defined as the concentration required to inhibit the binding of labelled hCG by 50%, were compared: a peptide consisting of α-subunit residues 26–46 was found to be the most effective inhibitor (binding constants: α-26–46 10^{-4} M, α-subunit 10^{-7} M, intact hCG 10^{-11} M). It was also found to block the activity of thyroid-stimulating antibodies. This suggests that the binding site for α-26–46 is close to the immunogenic site recognized by autoantibodies from patients with Graves' disease. A weaker inhibitor region was found at α-76–92 (binding constant $>10^{-3}$ M) (Ryan *et al.*, 1988). The model proposed by Moyle *et al.* (1987) has these two regions internally stabilized by the disputed disulphide bridges. By contrast, the model of Willey and Leidenberger (1989) does not have these binding 'loops' internally stabilized. However, in this model the two binding loops are linked by a disulphide bridge α-Cys-36–Cys-88.

The specific β-subunits of the intact hormones bind directly to receptors. Two major receptor-binding regions have been identified: β-Cys-93–Cys-100 and β-Cys-38–Cys-57. The β-Cys-93–Cys-100 region is common to all the glycoprotein β-subunits and has a binding constant of 10^{-4} M (free β-hCG 10^{-4} M, intact hCG 10^{-10} M). Peptide β-Cys-38–Cys-57 has a similar binding constant (10^{-4} M) but is only common to β-hCG and β-LH. There are three amino acid differences in the primary structure of LH in this region; this is reflected in the slightly greater binding constant for the LH-derived peptide (approximately 10^{-5} M). Thus, this region may represent the hCG/LH-specific receptor-binding determinant (Ryan *et al.*, 1987, 1988). Monoclonal antibodies raised against these receptor-binding regions inhibit the assembly of the intact hormone. Thus, the combined receptor-binding/stimulating region of intact

hCG appears to be at the interface between the α and β-subunits (Bidart *et al.*, 1988; R.K. Campbell *et al.*, 1988; Canfield *et al.*, 1988; Ryan *et al.*, 1988).

Quaternary CG structure and relationship to serine proteases

In the sequence analysis and three-dimensional structural models of Willey and Leidenberger (1989), α- and β-subunit shape was largely based on the similar core structure of chymotrypsin. In chymotrypsin, and all serine proteases, enzymatic activity is a tertiary interaction of remote linear sequence amino acids, serine, histidine and aspartic acid. By homologous modelling, Willey and Leidenberger (1989) suggested that, for hCG, Ser-38 and His-83 on the α-subunit formed a similar active site with the β-subunit Asp-99, the latter being the only conserved aspartic acid residue in all reported β-subunit sequences (Bousfield *et al.*, 1987). Recently, Chen and Puett (1992) changed the hCG β-subunit Asp-99 to an arginine residue by site-directed mutagenesis.

Co-transfection with a bovine α-subunit gene into CHO cells resulted in expression of an intact CG molecule which bound to the LH–CG receptor. However, it could not trigger cAMP production and thus biological activity was abolished. No other mutations were present and similar constructs using wild-type hCG β-subunit gene were effective stimulators of cAMP production. The evidence is that a serine protease-like interaction of hCG α-Ser-38, α-His-83 and β-Asp-99 activating the LH–hCG receptor would imply that cleavage of peptide bonds causes an irreversible change in the conformation of the triggered receptor. Furthermore, this interaction would be common to all glycoprotein hormones when activating their respective receptors. Such a mechanism might have evolved by retaining tertiary/quaternary structures for core cleavage function (receptor activation), whilst the addition of new structures conferred specificity (i.e. receptor binding).

5.3.4 CONTROL MECHANISMS

Regulation of the secretion of hCG by the placenta is not well understood. LHRH-like material has been identified in placental secretions, and it is possible that there is control similar to that of pituitary hormones (Belisle *et al.*, 1986; Elkind-Hirsch *et al.*, 1989). In support of this, Siler-Khodr *et al.* (1983) showed that an antagonist to LHRH inhibited the release of hCG, α-subunit and progesterone from mid-term placentas *in vitro*. This effect occurred rapidly and could be partially restored by LHRH. Gibbons *et al.* (1975) demonstrated that trophoblastic cells synthesize LHRH *in vitro*. There is *in vitro* evidence that progesterone suppresses the release of hCG (E.A. Wilson *et al.*, 1980, 1984a) but there is little information on how progesterone or other factors modulate cellular levels of mRNAs in coding α- and β-hCG. Maruo *et al.* (1986) investigated the effect of progesterone on the production and secretion of hCG in normal placenta and choriocarcinoma. In normal early placentas, progesterone decreased cellular levels of α- and β-hCG mRNAs and there was a fall in the hCG and α- and β-hCG released. Progesterone did not affect hPL mRNA or hPL release. However, choriocarcinoma tissue did not respond to progesterone.

Several other agents modulate hCG secretion. Epidermal growth factor (EGF) can increase DNA, RNA and protein synthesis in cells. Receptors for EGF have been identified in many tissues, including the placenta. EGF does not increase hCG production from normal placentas but doubled the secretion from a choriocarcinoma cell line (Huot *et al.*, 1981; E.A. Wilson *et al.*, 1984b). The placenta is also rich in adrenergic receptors and β-adrenergic-stimulated adenylate cyclase. Shu-Rong *et al.* (1982) found that the adren-

ergic β-receptor agonist terbutaline increased placental hCG secretion but did not affect hPL. Corticosteroids have also been implicated. E.A. Wilson and Jawad (1982) observed that natural and synthetic corticosteroids increased the secretion of hCG from placental tissue *in vitro* and the effect was decreased by progesterone: hPL secretion was not found to be affected by corticosteroids.

Retinoic acid and synthetic analogues of retinoic acid control cell differentiation in many epithelial tissues, and these were found by Chou *et al.* (1983) to induce placental hCG secretion *in vitro* while inhibiting α-hCG secretion. The ratio of α-hCG to intact hCG was also altered in choriocarcinoma cells.

5.3.5 BIOLOGICAL PROPERTIES

Strong circumstantial evidence has dictated the 'classical' function of hCG as the human fetal/placental signal for maternal recognition of pregnancy. In any animal in which the gestational period exceeds that of the oestrous cycle, the conceptus must signal its presence at implantation, halt ovarian cyclicity and maintain the receptive endometrium by rescue of the corpus luteum. This process is essential in many placental mammals. CG is known to have gonadotrophic activity (Aschheim and Zondek, 1927b) and, by virtue of its close structural and genetic homology with LH, it binds and stimulates the LH receptor. Not surprisingly, in primates and equids, CG is the suggested luteotrophic mediator of this process. In early human pregnancy the corpus luteum is the major source of the progesterone, which maintains the thickened endometrium (Csapo and Pulkkinen, 1978). During a normal human menstrual cycle, progesterone levels rise rapidly for the first 6–7 days following ovulation, plateau for 3–4 days then decrease until menses. In pregnancy, by contrast, the corpus luteum continues to produce progesterone for at least 4–6 weeks after ovulation (Tulsky and Koff, 1957). Thereafter it becomes relatively quiescent: at 6–8 weeks progesterone levels transiently decrease, followed by a secondary rise with the independent secretion of progesterone by the placenta (the 'luteal–placental shift') (Batzer, 1980).

There is much evidence to support the view that CG is responsible for the rescue of the corpus luteum. It is secreted very early in pregnancy, being detectable in culture media of preimplantation human embryos 7–8 days after *in vitro* fertilization, and in maternal serum 6–9 days after conception (Braunstein *et al.*, 1976; Fishel *et al.*, 1984; Lopata and Hay, 1988). The progesterone rise during the first 4 weeks following conception follows the rise in maternal serum hCG (Yoshimi *et al.*, 1969). Cultured luteal cells from different stages of development of the corpus luteum show peak hCG/LH receptor concentrations at 20–24 days post menses (Bolton *et al.*, 1980). This coincides with the earliest time at which maternal serum hCG can be detected.

In vitro, hCG stimulates luteal cell production of progesterone and oestradiol (Marsh and LeMaire, 1974; Richardson and Masson, 1981). *In vivo*, administration of hCG can increase the amount and duration of progesterone secretion (Hanson *et al.*, 1971). However, the menstrual cycle is rarely prolonged for more than 2 weeks despite continued administration of hCG (Bradbury *et al.*, 1950). In addition, corpus luteum function wanes and the luteal–placental shift occurs when maternal hCG reaches its peak, i.e. 8–12 weeks. Furthermore, it is somewhat surprising that such a fundamentally important function is mediated in primates by a recently evolved gene only found in this genus (Fiddes and Talmadge, 1984). It is now clear that in ruminants maternal recognition of pregnancy is mediated via a member of the alpha-interferon (IFN-α) family of proteins and is produced by the trophectoderm (Imakawa *et al.*, 1987; Flint *et al.*, 1988). It has still to

be determined whether IFN-α-like proteins also mediate corpus luteal rescue in other non-primate mammals. Interestingly, IFN-α is also a product of the human conceptus and *in vitro* IFN-α increases ectopic expression of free β-hCG by epithelial tumours (Chard *et al.*, 1986; Iles and Chard, 1989a).

Though other early pregnancy factors may play a role in the rescue of the corpus luteum of pregnancy, it appears that CG is essential in early primate and human pregnancy and directly implicated in corpus luteum function. Passive immunization of pregnant marmoset monkeys with anti β-hCG serum results in spontaneous abortion in early pregnancy (reviewed by Hearn, 1976; Chard, 1991). This can be counteracted by the concomitant administration of progesterone. Such experiments have led to the development of experimental contraceptive vaccines using synthetic peptides analogous to the specific carboxy-terminal sequence of hCG (Stevens and Crystle, 1973; Stevens, 1975, 1986).

HCG has also been implicated in the suppression of maternal immune rejection of the fetus. Generalized immunodeficiency is not a characteristic of pregnancy (reviewed by Redman, 1986). Specific immunosuppressive activity has been actively sought and many substances have been implicated, including hCG (reviewed by Hogarth, 1982; Chard and Grudzinskas, 1992). The literature contains many conflicting reports; in general, crude but not pure hCG has immunosuppressive activity on T-cell and B-cell activities *in vitro*. Indeed, molecules of low molecular weight, including preservative phenol, have been separated from crude commercial preparations of hCG and have been shown to be the cause of apparent immunosuppression (Morse *et al.*, 1976; Pattillo *et al.*, 1976; Muchmore and Blaese, 1977). *In vivo*, highly purified hCG suppresses delayed-type hypersensitivity. However, this could be entirely attributed to the gonadal steroids secreted in response to hCG, since castrated experimental animals showed no such immunosuppression (Nisula and Bartocci, 1984).

5.3.6 RECEPTORS

The LH–hCG receptor has been identified in many cells, including those of the corpus luteum, the interstitial tissue of developing follicles and in the granulosa as well as in Leydig cells in the testis. There are about 20 000 sites per Leydig cell and only a minute fraction of the total number need be occupied to produce a full steroidogenic effect (Dufau *et al.*, 1974). Only intact hCG and LH, but not the free subunits, can bind to and stimulate the LH–hCG receptor. The sugar residues of these glycoprotein hormones are essential if this receptor is to be successfully triggered (Keene *et al.*, 1989). The immediate consequence of successful receptor stimulation by hCG is the activation of adenylate cyclase, resulting in the formation and increase in concentration of intracellular cAMP. This secondary signalling culminates in the stimulation of steroid production by the acceleration of the conversion of cholesterol to pregnenolone. The LH–hCG receptor has been cloned and found to be a member of the G protein-coupled receptor family (Loosfelt *et al.*, 1989; McFarland *et al.*, 1989). There are 674 amino acids with a calculated molecular mass of 75 632 kDa, but the *in vivo* isolated protein has a mass of approximately 93 000 kDa. This difference is probably due to glycosylation, for which there are six candidate sites located on the transmembrane domain. The molecule is asymmetrical with classic extra-, trans- and intracellular domains. The structure and function have been reviewed by Dias (1992).

5.3.7 DETECTION AND MEASUREMENT OF hCG

From 1930 until 1960 the detection of hCG in biological fluids depended on bioassay. Many methods were variations on the hyper-

ovulatory response in animals shown by Aschheim and Zondek (1927b). Aschheim and Zondek (1928) used mice; Friedman and Lapham (1931) rabbits; Shapiro and Zwarenstein (1934) toads; Wiltberger and Miller (1948) frogs. Others relied on effects such as the stimulation of other organs by gonadal steroids, e.g. the rat ventral prostate weight assay (Diczfalusy *et al.*, 1950). The same principle is used in current bioassays, in which direct measurement of testosterone release is determined for rat Leydig cells stimulated *in vitro* (Dufau *et al.*, 1974). With the possible exception of the *in vitro* Leydig cell assay, bioassays are expensive, time-consuming (11 h to 7 days), relatively insensitive (250–5000 iu/ml) and non-specific with respect to hCG, LH and FSH. The development of immunoassays in the 1960s (complement fixation, haemagglutination inhibition and latex agglutination procedures) improved assay times (2 min to 1 day) and reduced costs. Nevertheless, sensitivity was no better (500–24 000 iu/ml) (Wide and Gemzell, 1960, reviewed by Hussa, 1987). Competitive binding assays such as radioimmunoassay (RIA) and radioreceptor assays solved the sensitivity problem. However, because of the close homology of hCG and LH there was still significant cross-reactivity with the latter (Catt *et al.*, 1971). Dissociation of the hCG molecule into subunits and preparation of antisera to the free β-subunit led to the development of the much more specific 'β-subunit' RIA (Vaitukaitis *et al.*, 1972). This allowed specific measurement of hCG in the presence of physiological levels of LH. These assays have been the mainstay of quantitative detection technology for hCG since the mid-1970s. The problem of false-positive results with various kits is still reported from time to time and, therefore, there has been increasing use of more specific systems. Rzasa *et al.* (1984) found five false positives in 162 samples investigated with one commercial kit designed to measure intact hCG or β-hCG using a polyclonal antibody, but not with three other similar kits; no obvious reason for the discrepancies could be found. Filstein *et al.* (1983) found 0.6% false positives with two kits based on serum β-hCG assays but not with a third.

A further advance came with the application of monoclonal antibody technology to two-site immunometric assays (Bidart *et al.*, 1985; Schwarz *et al.*, 1985; Valkirs and Barton, 1985). Typically, these use an anti α-subunit monoclonal for capture and a labelled anti-β-subunit for detection; they detect intact hCG but not free subunits. These labelled antibody assays are generally more sensitive and precise than labelled ligand assays. However, the inability of these assays to detect free β-hCG is, as will be shown later, a major disadvantage in clinical oncology.

Ehrlich *et al.* (1982) used a mixture of two monoclonal antibodies to enhance the affinity for antigen. Armstrong *et al.* (1984) developed this idea to obtain maximum sensitivity and specificity in a two-site immunoradiometric (IRMA) system. hCG was extracted from urine by a Sepharose 4B-coupled monoclonal antibody which had a moderate affinity ($K=7 \times 10^8\ M^{-1}$) and 2% cross-reaction with LH. The hCG extracted was then determined by use of a labelled rabbit antibody to the β-hCG C-terminal peptide. The method was capable of measuring about 10 miu of hCG per 24 h with cross-reaction to LH of only 0.0005% and to FSH of 0.016%, possibly due to the presence of small amounts of hCG or β-subunit in the pituitaries from which the hormones were extracted. Another IRMA using two selected monoclonal antibodies was specific for intact hCG and showed no reaction with β-hCG, β-LH, intact LH or the free α-subunit (Griffin and Odell, 1987).

It is now very important to define exactly what is measured by 'hCG' immunoassays. Assays based on the use of an antiserum to the β-subunit of hCG are commonly referred to as 'beta-subunit' assays. Vaitukaitis (1985) pointed out that this is misleading because it implies that only β-hCG is measured when,

in fact, intact hCG, β-hCG and β-core may be detected. Intact hCG is the predominant form of the hormone in blood during pregnancy. However, for many cases of ectopic expression free β-subunit is the principal form produced (Iles and Chard, 1991) and in urine β-core is the predominant metabolite. This limits the use of many highly specific assays. Thus, only those 'β-subunit' assays measuring 'total β-hCG' (intact hCG, free β-subunit and β-core) should be used in oncology. This problem has been addressed by the Fourth Bergmeyer Conference on the Standardization of Assays to hCG. This recommended clear description of all hCG assays detailing the cross-reactivities of intact hCG, free subunits and β-core (Stenman *et al.*, 1993).

(a) Reference standards

The first international standard for hCG was established in 1938 and the second in 1964 by the World Health Organization (WHO) (Bangham and Grab, 1964). The standard preparation is of medium potency prepared from urine by adsorption on to the ion exchanger Permutit (synthetic aluminium silicate), followed by elution in a mixture of ammonium acetate and ethanol. After precipitation from ethanol, pyrogens are removed by adsorption on to calcium phosphate. Lactose is added and the preparation is freeze dried in ampoules; the agreed potency for each ampoule (approximately 6.8 mg) is 5300 IU as determined by bioassay.

The second international standard (2nd IS hCG) was used widely as a reference standard for immunoassays as well as bioassays. However, it contains large quantities of the free subunits and other proteins, being only 20% pure intact hCG on the basis of bioactivity (Bangham and Storring, 1982).

In 1974, large quantities of highly purified preparations of intact hCG, free α-subunit and free β-subunit hCG (batch no. CR119) were donated to the WHO by the Center for Population Research (Washington DC, USA) and the Reproductive Research Branch of the National Institute of Child Health and Human Development (Bethesda, MD, USA). These preparations became the international reference preparations (IRPs) of intact hCG and its free subunits, each ampoule containing approximately 70 μg of the pure material (Storring *et al.*, 1980). These IRPs were assayed with respect to the old 2nd IS hCG by bioassay and immunoassays. Bioassay and receptor-binding assays (which only detect intact hCG) gave similar results. However, immunoassay results varied widely depending on whether the assay measured only intact hCG or intact hCG and free β-subunit.

The immunoassays specific for intact hCG gave results similar to those obtained for bioassays. The Expert Committee on biological standardization assigned a value of 650 IU of IRP per ampoule (9.3 IU per μg) of intact hCG based on bioassay comparability with the earlier standards. The free subunits, having no bioactivity, were assigned IU values equivalent to the mass of purified protein (i.e. 1 IU = 1 μg). This situation has proved confusing since most present assays of hCG are immunoassays measuring mass of specific epitopes. Unfortunately, all such assays use the intact hCG IRP or 2nd IS as standards. In total β-subunit assays pregnancy serum hCG values measured against the 2nd IS are approximately half that of values measured against the IRP hCG. This is simply because the 2nd IS is contaminated with large amounts of the free β-subunit, resulting in greater immunopotency. Samples containing the equivalent IU of intact hCG though very little free β-subunit (i.e. the IRP) will have the same biopotency but less immunopotency. It is also increasingly apparent that there is a wide variation in the composition of intact and free subunits of hCG in clinical samples (Iles *et al.*, 1989, 1992). It has now been recommended that hCG standards should be calibrated in terms

of moles of intact hCG, free β-hCG or β-core (Stenman *et al.*, 1993).

5.3.8 CLINICAL APPLICATIONS – DIAGNOSIS

(a) Maternal levels of hCG in normal pregnancy

Chorionic gonadotrophin is detectable in maternal blood within a few hours of implantation. The levels rise to reach a peak at 60–80 days of pregnancy and then fall to relatively low levels with a small secondary rise towards term. In early pregnancy (up to 28 days) the doubling time is 1.4–1.5 days, increasing to 2.7–2.9 days at 4–8 weeks' gestation (Pittaway *et al.*, 1985a). At term, the levels of hCG in blood, amniotic fluid and placental extracts are substantially higher if the fetus is a female than if it is a male (Brody and Carlstrom, 1965; Crosignani *et al.*, 1972; Wide and Hobson, 1974; Boroditsky *et al.*, 1975; Obiekwe and Chard, 1983a). Urine of pregnant women contains free α-subunit, free β-subunit, hCG and the β-subunit core fragment (Blithe *et al.*, 1988). Not surprisingly, the ratio of the biological to immunological activity of hCG varies at different stages of pregnancy (Kato and Braunstein, 1988) with low ratios in the last two trimesters.

(b) Measurement of hCG as a pregnancy test

Measurement of hCG in urine is widely used as a qualitative test of early pregnancy. Simple kits based on immunoenzymatic procedures are available over the counter. Their sensitivity (typically 25 IU/l) is sufficient to detect most pregnancies at or around the time of the missed period, and a substantial proportion at an earlier stage than this.

The high sensitivity of current technology for hCG measurement leads to two problems. First, at levels below 5 IU/l positive results may occur due to hCG from extrauterine sources (Armstrong *et al.*, 1984); in clinical practice, a single estimate of hCG levels should only be considered positive if it is greater than 25 IU/l or if a lower level is seen to increase twofold or more at an interval of 3 days (Jones *et al.*, 1983). Second, there is a highly significant possibility that a sensitive test may pick up an 'occult' pregnancy. This is a conception which is aborted after implantation but prior to the time of the expected period: there may be no clinical evidence for this situation but hCG can appear transiently in maternal blood and urine. The incidence of the phenomenon has been variously estimated at 15–60% of normal unprotected menstrual cycles (Seppala *et al.*, 1978; Chartier *et al.*, 1979; Miller *et al.*, 1980; Edmonds *et al.*, 1982; R.G. Edwards and Steptoe, 1983; Jones *et al.*, 1983; Whittaker *et al.*, 1983; Wilcox *et al.*, 1985; Sharp *et al.*, 1986; Chard, 1991). Transient appearance of hCG has also been shown in women using an intrauterine contraceptive device (Beling *et al.*, 1976; Landesman *et al.*, 1976; Hodgen *et al.*, 1978).

Measurement of hCG for estimation of gestational age

A single estimation of hPL, hCG or Schwangerschafts-protein 1 (SP1) can be used to estimate gestational age at 4–6 weeks of pregnancy (Whittaker *et al.*, 1983; Lagrew *et al.*, 1984; Westergaard *et al.*, 1985b). In practice the error range is similar to that which can be obtained from an accurate menstrual history or an ultrasound scan.

Threatened abortion

The levels of all placental products, including hCG, are reduced in cases of threatened abortion which subsequently proceed to complete abortion (Nygren *et al.*, 1973; Braunstein *et al.*, 1978; Jovanovic *et al.*, 1978; Jouppila *et al.*, 1979; Masson *et al.*, 1983; Salem *et al.*, 1984b; Yovich *et al.*, 1986). As a clinical test this measurement has been largely superseded by

ultrasound; demonstration of the absence of a fetal heart beat gives the same information more simply and rapidly. However, serial measurements of hCG (i.e. showing failure of the normal rapid rise) may be of value in the period prior to the appearance of the fetal heart beat (Yovich *et al.*, 1986).

Ectopic pregnancy

Circulating hCG is usually present in cases of ectopic pregnancy, and measurement of hCG in blood and/or urine has been proposed as a routine test in all cases of lower abdominal pain in women of reproductive age (Seppala *et al.*, 1980; Braunstein and Asch, 1983; Norman *et al.*, 1985b). A negative result (less than 25 IU/l) virtually excludes the possibility of a pregnancy-related disorder (Romero *et al.*, 1985a). Quantitative estimations of hCG may also be of value, especially in combination with ultrasound examination. A level of hCG greater than 6500 IU/l, with no evidence of a live intrauterine embryo, is particularly suggestive of ectopic gestation (Pittaway *et al.*, 1985b; Romero *et al.*, 1985b).

Fetal congenital abnormality

Trisomy 21, Down's syndrome, is the most common autosomal aneuploidy in which affected individuals survive beyond infancy. The risk of a Down's syndrome pregnancy increases with maternal age, and pregnant women over 35 are routinely offered amniocentesis. This accounts for only 25–30% of all cases of Down's syndrome, representing only 5–7% of the total pregnancy population. During the second trimester, maternal serum α-fetoprotein (AFP) levels are lower in cases of Down's syndrome than normal affected pregnancies (Merkatz *et al.*, 1984). In addition unconjugated oestriol (uE3) is also decreased (Canick *et al.*, 1989) and human chorionic gonadotrophin (hCG) levels are about double those of normal pregnancies (Bogart *et al.*, 1987). Currently the best predictor of Down's syndrome during the second trimester of pregnancy is the combination of elevated hCG, low levels of AFP and low levels of uE3. This 'triple' test can detect about 60% of all fetal Down's syndrome with an amniocentesis rate representing about 5% of the total pregnant population (Wald *et al.*, 1988).

Though this represents a great advance in prenatal diagnosis of Down's syndrome, definitive diagnosis is late. Other markers are being investigated in order to improve the detection rate and decrease the gestational age at which the test is effective. Levels of the free β-subunit of hCG appear to be elevated in the maternal serum of Down's affected pregnancies during the first trimester (Spencer *et al.*, 1992). Furthermore, the measurement of free β-hCG appears to be a superior discriminant of Down's pregnancies than measurement of intact and total β-hCG. Measurement of maternal free β-hCG and AFP is replacing the triple test as the preferred biochemical screening test for Down's syndrome.

(c) Maternal levels of hCG in complications of late pregnancy

By comparison with other placental products, such as hPL and oestriol, there is a very limited literature on hCG levels in complications of late pregnancy. The current evidence indicates that serum hCG levels are higher in primigravidas and in women carrying female fetuses, and that they are also related to birthweight (Obiekwe and Chard, 1982a, 1983a). The levels are elevated in primigravid patients with proteinuric pre-eclampsia (Said *et al.*, 1984). Amniotic fluid and cord blood levels of hCG are elevated in diabetic mothers (Barbieri *et al.*, 1985, 1986).

(d) hCG as a tumour marker

The value of detecting in hCG in clinical samples from patients with trophoblastic disease was recognized soon after its discovery

(Aschheim, 1930). The same bioassay systems were used to detect and monitor the first reported cases of ectopic hCG production by non-trophoblastic tumours (MacFadzean, 1946; Chambers, 1949). Gestational and male testicular germ cell tumours are the best-known categories in which ectopic hCG expression occurs. Monitoring this expression has proved invaluable in the diagnosis and treatment of these once lethal tumours. The third category, non-trophoblastic neoplasms, is the most controversial area of ectopic hCG expression.

Gestational trophoblastic disease

The most efficient marker of any cancer is hCG production by gestational choriocarcinoma and hydatidiform mole. hCG has provided a reliable monitor of staging, course of disease and response to chemotherapy for over 40 years. High pretreatment levels correlate with poor prognosis, i.e. between 1000 and 10 000 μIU/ml are associated with a 2.5% mortality rate, while values exceeding 1 million are associated with a 60% mortality rate (Hussa, 1987). Following treatment, levels are determined every 1–2 weeks. As long as these decrease to within normal limits second line treatment is withheld. Remission is defined on the basis of serum hCG levels of < 5 μIU/ml for 3 consecutive weeks. The policy of the Charing Cross Reference Centre is to continue monitoring every 2 weeks up to 6 months then monthly for 5 years (Bagshawe, 1980, 1984; Hussa, 1987).

Though chemotherapy regimens vary between centres, the levels of hCG determine when and how the therapy is administered. High-level, high-risk, categories receive more neoadjuvant courses once remission is achieved than low-risk groups. In addition, in the absence of clinically apparent disease, persistently elevated serum hCG reflects the existence of tumours that if untreated will progress clinically (Hussa, 1987). In some cases, differential levels of hCG in clinical samples can pinpoint the site of metastases (e.g. a serum–CSF ratio of greater than 60:1 is indicative of spread to the brain) (Soma *et al.*, 1981; Bagshawe, 1984; Hammond *et al.*, 1984).

The hCG found in serum from patients with gestational tumours is predominantly intact hCG. Elevated levels of free α-subunit are also found. Vaitukaitis and Ebersole (1976) suggested that elevated free α-subunit correlates with poor prognosis, in particular chemoresistance. Quigley *et al.* (1980a,b) also suggested that elevated serum α-subunit during remission may identify patients in whom recurrence occurs. Gaspard *et al.* (1980) found that free α-subunit levels were similar to those in pregnancy of similar gestational age. Analysis of the ratio of intact to free β-hCG has shown that in choriocarcinoma there is a greater proportion of free β-subunit than in hydatidiform moles or normal pregnancy (Gaspard *et al.*, 1980; Khazaeli *et al.*, 1989). Not surprisingly, high levels of β-core are detected in the urine of patients with gestational disease. Choriocarcinoma hCG tends to be hyperglycosylated and the extent of this is believed to correlate with malignant behaviour (Kobata, 1988).

Testicular germ cell tumours

More than 95% of all testicular tumours are of germ cell origin. There are two major types: seminomatous and non-seminomatous (or teratocarcinomas). The non-seminomatous tumours are of five histological subtypes: embryonal carcinoma, teratocarcinoma, teratoma, choriocarcinoma and yolk sac carcinoma. It is unusual to find a single histological type in a diseased testis. Even seminoma and non-seminomatous elements can be found simultaneously and a single random biopsy can prove misleading (Caulfield *et al.*, 1990). The frequency of hCG expression varies from 8–9% for pure seminomas to 100% for pure choriocarcinomas. Non-seminomas are more likely to express hCG than seminomas. Non-

seminomatous tumours are sometimes divided into mature or immature teratomas. The mature teratomas contain highly differentiated tissues such as cartilage and bone: whilst the immature forms contain predominantly developmental tissues such as syncytiotrophoblast and yolk sac elements. The oncofetal antigen α-fetoprotein (AFP) is also produced by some testicular germ cell tumours. Unlike hCG, AFP expression is diagnostic of non-seminomatous disease as it is a specific product of the yolk sac. This diagnostic distinction is critical since seminomas are radiosensitive whilst non-seminomas only respond to chemotherapy. Furthermore, incorrect treatment of non-seminomatous tumours with radiotherapy invariably precludes subsequent chemotherapy. As with gestational trophoblastic disease, hCG levels at presentation are prognostic. Patients with levels greater than 10 000 μIU/ml have a poorer 3-year survival rate (53%) than those with levels less than 1000 (85%) (MRC Working Party on Testicular Cancer Report, 1985). Chemosensitivity is a predominant feature of hCG-expressing non-seminomatous tumours and the cure rate is high.

The hCG present in patients with non-seminomatous testicular germ cell tumours is similar to that of choriocarcinoma: predominantly intact with varying amounts of free β-subunit (Norman *et al.*, 1985c). hCG expression by seminomatous disease may also indicate the presence of trophoblastic elements. The overall survival rate for seminomatous disease is 80–97% (Schuette *et al.*, 1985; Thomas, 1985). However, for hCG-positive seminomas there is a 20% mortality rate at 2 years (non-expressors 4%) and a reported correlation with radioresistance (D.A. Morgan *et al.*, 1982; Butcher *et al.*, 1985). Though testicular germ cell tumours predominantly express intact hCG, there are reports of cases in which only free β-hCG is expressed (Light *et al.*, 1983; Rzasa, 1984; Hussa, 1987). Hussa (1987) has estimated the incidence of pure β-hCG-expressing testicular germ cell tumours as 2–4%. However, Saller *et al.* (1990) have reported that in seminomas the predominant species is free β-hCG.

Ectopic expression by non-germ cell tumours

Prior to the development of the β-subunit RIA, studies of ectopic hCG production by non-trophoblast non-germ cell tumours was limited to sporadic case reports. The most frequent sites were lung, liver (hepatoma) and the adrenal cortex (reviewed by Braunstein, 1983). After the development of the 'β-hCG' RIA, Braunstein *et al.* (1973) published the seminal study on expression of hCG-like material by non-trophoblastic cancers. Eleven percent of 906 patients with various non-trophoblastic malignancies had immunoreactive hCG in their serum. Many subsequent reports have given a varying incidence for specific tumours. Baylin and Mendelsohn (1980), Heyderman *et al.* (1985) and Seppala *et al.* (1986) generally agree that the most common occurrence of immunoreactive hCG expression is in pancreatic (50%), gastric (24%) and hepatic (17%) tumours. However, most studies are case reports. Braunstein (1983) and Hussa (1987) both reviewed the literature in detail. Their estimated prevalence of hCG for various tumours is shown in Table 5.2.

Though there are some discrepancies between tumour types, both authors agree that the overall prevalence is low (19–21%), that the levels are usually between 5 and 25 μIU/ml, and that if the criteria for ectopic expression were levels of 25 μIU/ml or greater the overall prevalence would be 1–5% (Braunstein, 1983; Hussa, 1987).

There are technical problems with the assessment of low levels of hCG. The range 5–25 μIU/ml is at the lower and least reliable end of most assays. Several studies have shown that protease activity in clinical samples may give false-positive results (Adejuwon *et al.*, 1980; Hussa *et al.*, 1985). Variant

Table 5.2 Frequency of immunoreactive 'hCG' detection in sera of patients with non-trophoblastic neoplasms

Neoplasm	*Review*	
	Braunstein (1983)	*Hussa (1987)*
Ovarian (epithelial)	39% (101/260)	45% (97/216)
Gynaecological (non-ovarian)	27% (182/686)	32% (248/766)
Breast	26% (336/1311)	25% (167/667)
Gastrointestinal	21% (251/1211)	21% (163/768)
Pancreas	21% (31/150)	38% (23/50)
Head and neck	21% (17/83)	79% (19/24)
Melanoma	20% (38/194)	19% (40/120)
Lung	16% (187/1143)	15% (140/929)
Sarcomas	15% (11/76)	14% (7/51)
Renal	6% (7/18)	5% (7/128)
Prostate	7% (7/102)	10% (7/74)
Bladder	10% (5/48)	–
Multiple myeloma	8% (9/111)	7% (11/167)
Leukaemia–lymphoma	4% (29/814)	0.5% (23/545)
Total	19% (1175/6109)	21% (929/4455)

forms of hCG and subunits have been described in neoplastic isolates, and these may only have limited activity in a given assay (Hussa *et al.*, 1985). Immunoreactive hCG may be found in both tumour and normal tissues (Yoshimoto *et al.*, 1979); these studies are particularly prone to protease interference. Using assays of exceptional sensitivity and specificity Odell and Griffin (1987) have detected immunoreactive hCG at extremely low levels (0.4–7.9 μIU/ml) in postmenopausal serum. Subsequently, they described cyclical surges of hCG paralleling the LH surge during the menstrual cycle of non-pregnant women (Odell and Griffin, 1989).

McManus *et al.* (1976) described immunohistochemical detection of 'β'-hCG on the surface of nearly all (89%) of a group of malignancies. However, subsequent studies have not noted such a high frequency (Bellet *et al.*, 1980). Since β-hCG and intact hCG are rapidly secreted once formed, the specificity of these immunoperoxidase studies is questionable (Handwerger *et al.*, 1987). Given that only a few cases of ectopic hCG expression by non-trophoblastic tumours are genuine, the clinical relevance is also doubtful. Measurement of hCG has no value as a screening test. With gynaecological cancers the levels may parallel disease course (Tormey *et al.*, 1975; Broder *et al.*, 1977), but this is not always the case (Kahn *et al.*, 1977; Rutanen and Seppala, 1978; Stanhope *et al.* 1979; Carenza *et al.* 1980).

Despite the conflicting clinical information, *in vitro* studies have demonstrated hCG secretion by various non-trophoblastic tumours (Ruddon *et al.*, 1979a,b; Rosen *et al.*, 1980a; Iles *et al.*, 1990). Most only secreted one or other of the subunits; this contributed to the finding that the subunits of the glycoprotein hormones are coded by independent genes. It is now apparent that free β-hCG is also produced by many tumours arising from mucosal epithelia and various epithelial gynaecological neoplasms, including ovarian carcinomas (Braunstein *et al.*, 1973; Cole *et al.*, 1988; Cole and Nam, 1989; Iles *et al.*, 1990).

This expression is independent of the α-subunit (Iles and Chard, 1989a, Iles *et al.*, 1990).

Analysis of the genomic DNA structure of choriocarcinoma cell lines by Whitfield and Kourides (1985) showed no rearrangement of the α-subunit gene or the β-hCG–LH gene cluster which would result in aberrant expression. Using similar techniques and restriction enzymes, genomic DNA from β-hCG-expressing bladder tumour cell lines were similarly examined (Iles *et al.*, 1989). No gene rearrangement or amplification was found; furthermore, karyotype analysis did not show any consistent copies or translocations of chromosome 19. It therefore seems likely that such expression is a result of alteration in gene regulation/repression. However, normal urothelia and oral mucosa *in vitro* also secrete free β-hCG into their culture media (Iles *et al.*, 1990), and low levels of immunoreactive β-hCG can be detected in serum and urine of normal non-pregnant subjects (Chen *et al.*, 1976; Armstrong *et al.*, 1984; Huang *et al.*, 1984). It is therefore possible that free β-hCG is a normal product of mucosal epithelia with an as yet unknown function. Nevertheless, the detection of ectopic β-hCG expression by epithelial cancers correlates with a malignant phenotype of poor prognosis (J.E. Martin *et al.*, 1989; Iles and Chard, 1991; Marcillac *et al.*, 1992).

Which of the six β-hCG genes/pseudogenes is expressed by ectopic β-hCG-producing cancers has yet to be determined. At least five might be transcriptionally active. One or more might code for an aberrant form which cannot combine with the α-subunit (Bo and Boime, 1992).

5.3.9 CLINICAL APPLICATION – THERAPEUTIC

(a) Birth control vaccines

There has been much effort to produce a birth control vaccine which would act by neutralizing the action of hCG (WHO, 1985). Antibodies to hCG usually also recognize LH, and this would interfere with menstrual function. To achieve specificity, it is necessary to use the C-terminus of the β-subunit of hCG as the immunogen; this is not present in LH.

In earlier studies chemically altered forms of hCG or of hCG coupled to haptens were used as immunogens (Stevens and Crystle, 1973). Although it was possible to raise antibodies, they cross-reacted with LH and subsequent experiments addressed the β-subunit or fragments of the subunit. Hearn *et al.* (1976) showed that both active and passive immunization with the β-subunit could disrupt pregnancy. Active immunization also caused a marked reduction in the subsequent fertility of the animals. Other experiments with similar results were carried out with β-hCG coupled to tetanus toxoid in several species, including a limited number of women (Talwar *et al.*, 1976).

The optimal immunogen is the amino acid sequence 109–145 of the C-terminal region of the β-subunit of hCG with protein carrier and adjuvants acceptable to humans (Stevens, 1986). Tsong *et al.* (1985) found that the best results were obtained by linking the peptide via carbodiimide with tetanus toxoid. Numerous adjuvants were tried, of which the muramyl dipeptide family were most effective (Nash *et al.*, 1985), and the highest titres were obtained by suspending in saline and emulsifying with Arlacel A in squalene (C.C. Chang *et al.*, 1985). Preliminary clinical trials using this peptide have been carried out.

Most experimental work has used the baboon as a model (Stevens, 1976; Thanavale *et al.*, 1978). Stevens *et al.* (1981) however, found that the cross-reactivity between antibodies to the peptide immunogen and baboon CG was only 3–15%. Even then active immunization was effective in inhibiting fertility in these animals without side-effects.

5.3.10 CONCLUSIONS

Although a great deal has been learned in recent years about the structure and function of hCG, there remains much that is unknown. Study of the three-dimensional structure of the hormone by X-ray crystallography would be a significant advance. Complete sequencing of the entire β-hCG–LH gene cluster is required, as is elucidation of the transcriptional control mechanisms and the evolution of this gene cluster. The studies on structure–function relationship are still incomplete, and most conclusions are still hypotheses.

5.4 HUMAN PLACENTAL LACTOGEN

(a) Historical survey

The presence in the human placenta of a substance having mammotrophic activity was first clearly shown by Ito and Higashi (1961, 1974) and Higashi (1962). Subsequently, Josimovich and MacLaren (1962) demonstrated that the material was immunochemically similar to human pituitary growth hormone (hGH) and that it occurred in maternal peripheral serum, retroplacental serum and placental extracts.

(b) Nomenclature

There is much confusion over the nomenclature of the protein, which is referred to in this chapter as human placental lactogen or hPL. Many other designations have been put forward, including human chorionic growth hormone–prolactin (hCGP) (H. Cohen *et al.*, 1964; Grumbach and Kaplan, 1964), purified placental protein (PPP) (Florini *et al.*, 1966), human chorionic somatomammotrophin (hCS) (Li *et al.*, 1968) and choriomammotrophin (IUPAC-IAB, 1975). The term hCS is widely used and has been advocated by Bewley and Li (1974) on the grounds that it provides a clear description of the relationship of this protein to other hormones. Furthermore, the term 'chorionic' is more specific than 'placental' and is commonly used for other placental hormones such as chorionic gonadotrophin and chorionic corticotrophin. However, most workers consider the term chorionic somatomammotrophin to be cumbersome and inelegant and accordingly the original name of placental lactogen is now preferred.

5.4.1 BIOSYNTHESIS

(a) Source of hPL

As with other 'specific' placental proteins, hPL is a product of the syncytiotrophoblast, though there is evidence that in the very early placenta (4–5 weeks) the cytotrophoblast is the main source of both hPL and hCG (Maruo *et al.*, 1992). The experimental evidence for localization is based on immunohistochemistry (e.g. Ikonicoff and Cedard, 1973; Billingsley and Wooding, 1990; Maruo *et al.*, 1992) and studies with gene probes: using labelled complementary DNA ([^{3}H]cDNA) it has been shown that the bulk of the mRNA for hPL is in the syncytiotrophoblast (McWilliams and Boime, 1980; Sakbun *et al.*, 1990). When isolated cytotrophoblast cells are cultured *in vitro*, fusion and formation of a syncytium is associated with an increase in cytoplasmic hPL (Kliman *et al.*, 1986; Klassen *et al.*, 1989).

The localization of the protein-synthesizing machinery within the trophoblast has been studied by Burgos and Rodriguez (1966), who have shown a clear differentiation between thick areas of the trophoblast cytoplasm, which are rich in endoplasmic reticulum and microvilli and therefore appear to be specialized for protein synthesis, and thin areas without microvilli, which lie in proximity to fetal capillaries and appear to be specialized for the transport of nutrients and waste products. This differentiation is of potential clinical significance because it is possible that a

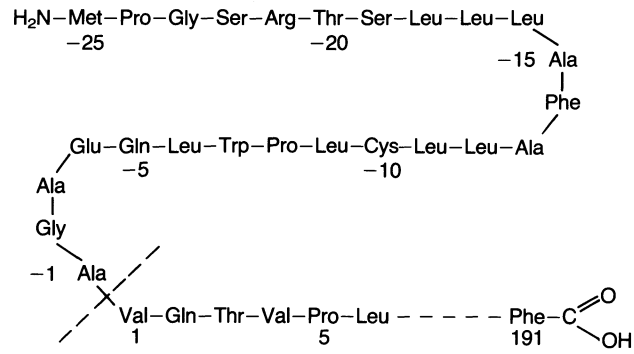

Figure 5.6 Amino acid sequence of the 25-residue 'precursor piece' at the *N*-terminus of hPL.

pathological process might damage the transfer area while leaving the synthetic areas intact. However, most trophoblast pathology is secondary to factors such as occlusion of fetal capillaries in the chorionic villus or maternal decidual arterioles, and it is likely in practice that all areas will be equally affected.

The distribution of hPL in different areas of the placenta appears to be homogeneous, the amount being directly related to the amount of trophoblast in any area (Gau and Chard, 1975). As already noted, production of hPL is not unique to the trophoblast: significant levels are found in seminal plasma (J.N. Lee *et al.*, 1983; Salem *et al.*, 1984a).

(b) Mechanism of biosynthesis of hPL

The basic mechanism of synthesis of hPL in the trophoblast appears to be similar to that of proteins in other tissues. Friesen and coworkers (Suwa and Friesen, 1969a,b) examined the incorporation of ^{3}H-labelled amino acids by placental fragments *in vitro*, and showed that hPL accounts for a substantial proportion (up to 55% in some experiments) of the total labelled protein released into incubation medium. In the tissue itself the ratio of labelled hPL to labelled total protein was much lower (4%), and most of the hPL appeared to be in a high molecular weight form (more than 100 000 daltons). The specific activity of labelled hPL was higher in the medium than the tissue, suggesting the existence of two pools in the latter site: one of rapid turnover and a second, more stable, variety.

Polysomes and messenger RNA from the placenta can direct the synthesis of hPL in cell-free systems (Boime and Boguslawski, 1974; Boime *et al.*, 1976; Cox *et al.*, 1976; Hubert *et al.*, 1981). Synthesis by polysomes from term placenta is four times greater than that of the first-trimester polysomes, and at term hPL represents 10% of the total proteins synthesized (Boime and Boguslawski, 1974). As the efficiency of the first-trimester and term mRNA is similar in terms of amino acid incorporation, it would appear that the relative levels of hPL mRNA are greater in the term placenta (Boime *et al.*, 1976). When free polysomes are used in a cell-free system a 'pre-lactogen' of molecular weight 25 000 is produced; the extra protein or 'precursor piece' is at the N-terminus of the molecule and contains an abundance of leucine residues (Birken *et al.*, 1977; Sherwood *et al.*, 1979). In the presence of membrane fractions from endoplasmic reticulum, the 'pre-lactogen' is converted to authentic hPL; this 'precursor piece' (Figure 5.6) is a 25 amino acid sequence which may play a part in the formation of membrane-bound ribosomal complexes (Blobel and Dobberstein, 1975) (Figure 5.7).

Thus, the primary gene product appears to

be pre-hPL with a molecular weight of 25 000 and it is likely that the high molecular weight material detected by Suwa and Friesen (1969a,b) represented a complex of pre-hPL and membrane components or possibly a stable dimeric form of hPL (two hPL molecules joined by disulphide exchange links) (Schneider *et al.*, 1977).

The genes for hPL and hGH synthesis are organized as a cluster at band q22–24 on the long arm of chromosome 17 and share a 92% sequence homology (George *et al.*, 1981; Selby *et al.*, 1984). There are five genes organized in the following manner: 5′-hGH-N, hCS-L, hCS-A, hGH-V, hCS-8-3′ (Harper *et al.*, 1982; Barsh *et al.*, 1983; Barrera-Saldana *et al.*, 1983). The mRNA products of these genes are heterogeneous, indicating multiple origins of transcription (Selvanayagam *et al.*, 1984). A transcriptional enhancer has been identified 2.2 kb 3′ of the hPL gene at the distal extreme of the hPL–hCG gene cluster (White *et al.*, 1993; Walker *et al.*, 1990). The inactive 'hCS-L' gene has a mutation at the 5′

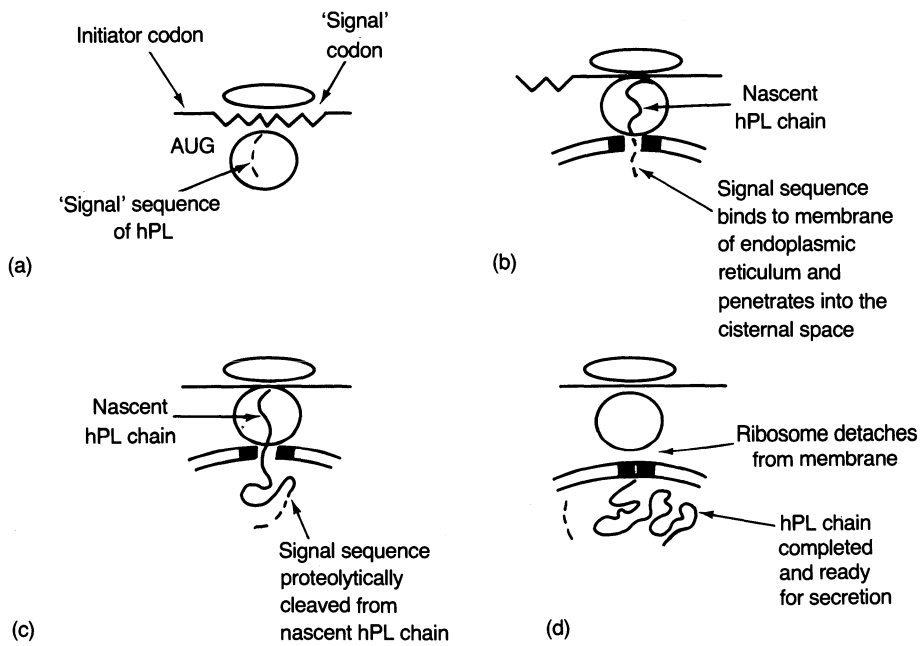

Figure 5.7 A scheme for the synthesis and secretion of hPL by the trophoblast, based on the experimental findings described in the text and the 'signal hypothesis' of protein secretion. (a) Synthesis of hPL is started by an initiator codon (AUG) on mRNA, together with two ribosome units. A unique sequence of codons, immediately to the right of the initiator codon is translated into a unique sequence of amino acids on the *N*-terminus of the nascent hPL molecule. This is the so-called 'signal sequence' of 'precursor piece', which contains a high proportion of the hydrophobic amino acid leucine. (b) The signal sequence binds to a receptor on the membrane of the endoplasmic membrane of the endoplasmic reticulum. This permits the nascent hPL chain to pass through the membrane into the cisterna. (c) The complete hPL chain enters the cisterna as it is translated by the ribosome (the ribosome remains bound to the membrane during this process). The signal piece is proteolytically cleaved from the nascent hPL chain. (d) The authentic hPL chain is completed and the ribosome dissociates form the membrane. This hypothesis explains why pre-hPL, i.v. with the signal sequence attached, is synthesized in cell-free systems containing only free polysomes, while authentic hPL is produced if a membrane fraction is included.

splice site, suggesting that it is a pseudogene (Hirt *et al.*, 1987). The hGH-V gene, previously thought to be inactive, is expressed in the placenta (Frankenne *et al.*, 1987). Varying numbers of gene deletions explain the rare cases of partial or total hPL deficiency (Parks *et al.*, 1985; Simon *et al.*, 1986). Some workers have suggested that the partial deficiency should be regarded as a reversion to the single-gene ancestral state rather than a gene deletion (Chakravarti *et al.*, 1984).

The cytological distribution of the mRNAs for hCG and hPL has been examined by *in situ* hybridization: hCG mRNAs are found partly in the syncytium and partly in the cytotrophoblast, while hPL mRNA is located exclusively in the syncytium (McWilliams and Boime, 1980; Hoshina *et al.*, 1982; Sakbun *et al.*, 1990). This association of hCG with differentiating elements and hPL with fully mature elements is compatible with the high levels of hCG and relatively low levels of hPL characteristic of trophoblast tumours (Goldstein, 1971).

(c) Distribution of hPL

The concentration of hPL in fetal blood is 100-fold less than that in the mother (Kaplan and Grumbach, 1965; Crosignani *et al.*, 1972; Houghton *et al.*, 1984). This differential is common to all specific proteins of the human placenta and is probably due to the fact that the trophoblast is in direct contact with the maternal circulation, whereas it is separated from the fetal circulation by a basement membrane and the fetal capillary endothelium. The concentration of hPL in the venous drainage of the uterus is similar or slightly higher than in the peripheral circulation (Klopper and Hughes, 1978; Grudzinskas *et al.*, 1979; Gusdon and Sain, 1981; Metzger *et al.*, 1985). The levels of hPL in amniotic fluid are 10-fold less than those in the mother (Crosignani *et al.*, 1972). Only small amounts of hPL are excreted in maternal urine (0.5 mg/day against a production rate of 1 g/day), a finding which is not surprising for a protein of this molecular weight. The concentration of hPL in mammary secretions during the last 12 weeks of pregnancy is approximately one-tenth of that in blood (Kulski *et al.*, 1982).

(d) Metabolism of hPL

The rate of synthesis of hPL has been estimated at 1–12 g/day (Beck *et al.*, 1965; Kaplan *et al.*, 1968). The half-life of hPL in the mother, as estimated by the rate of disappearance after delivery of the placenta, is 10–20 min (Spellacy *et al.*, 1966; Pavlou *et al.*, 1972). As with other biological molecules, the disappearance is biphasic with a rapid initial fall (yielding the half-life stated above) followed by a period of less rapid decrease. The differences in half-lives between individual subjects cannot be explained by observational error alone (Pavlou *et al.*, 1972). Each subject has her own characteristic half-life, and this can be attributed to differences in the mode of delivery and the physiological state of the patient.

There is no nyctohemeral rhythm in the maternal levels of hPL (Beck *et al.*, 1965; Samaan *et al.*, 1966; Ayala *et al.*, 1984). The variation over a 24 h period, however, is greater than that which can be attributed to the assay itself (Pavlou *et al.*, 1972); and this nyctohemeral variation is of the order of 6% (Houghton *et al.*, 1982). Thus, a single sample taken from an individual will not be representative of all samples taken from the individual, and the diagnostic significance of hPL levels is increased if serial levels are examined. The day-to-day variation of circulating hPL levels is similar to the nyctohemeral variation at 6% (Masson *et al.*, 1977). The factors responsible for the variation are unknown. Posture has no effect (Ylikorkala *et al.*, 1973), nor does strenuous physical exercise (Lindberg *et al.*, 1973; Pavlou *et al.*, 1973) or bedrest (Letchworth *et al.*, 1974), or a period of heavy smoking (Pavlou *et al.*, 1973). There is no change following the infusion of amino acids

or ingestion of a protein meal (Tyson *et al.*, 1969; Tatra *et al.*, 1977). Claims of fluctuations in levels during normal labour (e.g. Cramer *et al.*, 1971) have not been confirmed in other studies (Gillard *et al.*, 1973). There is no change in hPL levels associated with the onset of normal labour (Gillard *et al.*, 1973) or premature labour (Westergaard *et al.*, 1985a,b).

(e) Placental lactogenic hormones in other species

Protein hormones with lactogenic activity have been found in many but not all mammalian species (reviewed by Forsyth and Bloomfield 1986). Thus, it is found in the placenta of the goat (Buttle *et al.*, 1972; Currie *et al.*, 1977), the rhesus monkey (Walsh *et al.*, 1977), the sheep (Handwerger *et al.*, 1977), rodents (A.M. Kelly *et al.*, 1975), the hamster (Southard *et al.*, 1986, 1987), and the cow (Bolander and Fellows, 1976; Bolander *et al.*, 1976; Buttle and Forsyth, 1976). The most notable exceptions amongst mammals appear to be the rabbit, the pig and the dog (Porter, 1980). The placental lactogens of the chimpanzee, orang-outang and Old World monkeys are immunologically identical or very similar to hPL; the lactogen of the New World squirrel monkey, by contrast, shows only a marginal cross-reaction (Lin and Halbert, 1978). Similarly, there is virtually no immunological cross-reaction between the placental lactogens of the sheep, the goat, the cow and the deer, despite the close similarity of the prolactins and growth hormones in these species (Forsyth and Bloomfield, 1986).

Bovine placental lactogen (bPL)

The cow produces a 32-kDa protein with lactogen-like activity in radioreceptor assays (Eakle *et al.*, 1982; Byatt *et al.*, 1986, 1992). As with other ruminant hPLs, it is delivered to the maternal circulation by migration of binucleate cells of the placenta (Duello *et al.*, 1986; Byatt *et al.*, 1992). The serum levels of bPL remain low during the first two trimesters and thereafter rise rapidly to reach a plateau after 200 days (Bolander *et al.*, 1976). Dairy cows have higher bPL levels than beef cattle; the levels are higher in twin pregnancy but do not correlate with fetal weight or sex (Bolander *et al.*, 1976). Administration of recombinant bPL can increase milk yield and feed intake in the dairy cow (Byatt *et al.*, 1992).

Monkey placental lactogen

Placental lactogens are present in all groups of monkeys. In rhesus monkeys, PL is detectable from day 42 of gestation onwards (Walsh *et al.*, 1977). Maternal levels rise progressively and at term correlate with placental but not fetal weight (Novy *et al.*, 1981). The levels are not affected by protein deprivation, fetal anencephaly, or ligation of the interplacental bridging vessels (Novy *et al.*, 1981).

Mouse placental lactogen

The mouse placenta secretes two placental lactogens: mPL-I and mPL-II. Genes for both of these have been isolated and characterized (Jackson *et al.*, 1986; Shida *et al.*, 1992). Mouse placental lactogen (mPL) can be detected in maternal serum from day 9 of gestation onwards and its concentration increases throughout gestation (Soares *et al.*, 1982). The circulating levels correlate with litter size (Markoff and Talamantes, 1981; Soares and Talamantes, 1983). An increase in mPL levels is found after ovariectomy, hypophysectomy and fasting (Soares and Talamantes, 1985; Day *et al.*, 1986; Lopez *et al.*, 1991). Ethanol administration has no effect on mPL levels (Fielder *et al.*, 1989). *In vitro*, the release of mPL is principally a function of placental mass (Basch and Talamantes, 1986), though secretion of mPL-II can be inhibited by progesterone (Yamaguchi *et al.*, 1992). Some authors have concluded that mPL is an im-

portant lactogenic hormone in the second half of pregnancy in the mouse, when circulating mPRL levels are low (Thordarson *et al.*, 1986). It also appears to be lipolytic in this species (Fielder and Talamantes, 1987). Mouse placental lactogen I increases after day 8 to reach a peak at days 9–11, then declines (Ogren *et al.*, 1989).

Rat placental lactogen (rPL)

Like the mouse, the rat placenta secretes a number of prolactin-like molecules (M.C. Robertson *et al.*, 1982; Deb *et al.*, 1991; Kishi *et al.*, 1992), the levels of which increase in maternal serum during pregnancy. The source is trophoblast giant cells (Soares *et al.*, 1982), and a cDNA has been cloned (Duckworth *et al.*, 1986).

Adrenalectomy and unilateral oophorectomy have no significant effect on maternal rPL levels; bilateral oophorectomy or hypophysectomy leads to a substantial increase (M.C. Robertson *et al.*, 1984). There is an inverse relationship between rPL and rat prolactin during pregnancy (Voogt *et al.*, 1982); rPL may be responsible for the elimination of twice-daily prolactin surges during pregnancy in this species (Yogev *et al.*, 1983). Pregnant rats fed a low-protein diet have decreased levels of rPL and somatomedins, and the latter can be restored to normal by administration of hPL (Pilistine *et al.*, 1984). Administration of hPL can inhibit skeletal growth in young female rats (Chiang and Nicoll, 1992).

Sheep placental lactogen (oPL)

The sheep placenta secretes a placental lactogen with a molecular weight of approximately 23-kDa (Colosi *et al.*, 1989; Warren *et al.*, 1990; Byatt *et al.*, 1992). As with hPL a slightly larger form is synthesized (25-kDa) and a signal peptide is released as part of the secretions process. Ovine PL can be found in granules in trophoderm binucleate cells (Rice and Thorburn, 1986; Wooding *et al.*, 1992). Unlike the human, the hormone is present in substantial quantities in the fetal circulation as well as the maternal, though the levels in the fetus fall while maternal levels are still rising. The maternal levels are related to the number of fetuses present; the concentration reaches a peak at days 130–139 and thereafter falls to the time of delivery (Taylor *et al.*, 1980). Fetal plasma levels correlate with fetal weight (Schoknecht *et al.*, 1991).

In the sheep extensive metabolic studies have been possible. Intravenous administration of oPL to pregnant and non-pregnant sheep may result in a decrease in plasma free fatty acids, glucose, amino acids and insulin (Handwerger *et al.*, 1976). However, Thordarson *et al.* (1987) reported an increase in non-esterified fatty acids, glucose and urea during a continuous infusion of an ovine placental extract. *In vitro*, oPL stimulates glycogenesis by fetal hepatocytes (Freemark and Handwerger, 1986).

Administration of arginine or ornithine is a potent stimulus to oPL secretion (Handwerger *et al.*, 1981), as is infusion of high density lipoproteins (Grandis *et al.*, 1989). Acute hyper- or hypoglycaemia has little or no effect on oPL levels in mother and fetus, but fasting leads to a rise in oPL in both the fetus (Oliver *et al.*, 1992) and possibly the mother. Fasting of pregnant ewes causes a reduction in PL receptors in both the mother and the fetus (Brinsmead *et al.*, 1981; Freemark *et al.*, 1992). Infusion of arachidonic acid produces an increase in maternal but not fetal oPL levels (Huyler *et al.*, 1985). Administration of a 3β-hydroxysteroid dehydrogenase inhibitor, which causes a sharp fall in progesterone levels, has no effect on oPL secretion (Taylor *et al.*, 1982). Similarly, infusion of corticosteroids or corticotrophin has no effect on circulating oPL levels (Taylor *et al.*, 1983a); agents such as chlorpromazine and thyrotropin-releasing hormone, which produce a significant increase in fetal prolactin levels, have no effect on fetal oPL (Tay-

lor *et al.*, 1983b). Neutralization of oPL by *in vivo* administration of antibody to oPL appears to have no effect on glucose, fatty acids, somatomedins or growth hormone, but causes a fall followed by a rise in insulin levels (Waters *et al.*, 1985). *In vitro*, phospholipase C can stimulate oPL release (Rice and Thorburn, 1986).

Ligation of a single umbilical artery, which produces severe fetal growth retardation, is followed by a decrease in maternal levels of oPL but an increase in fetal levels (Newnham *et al.*, 1986a). Restriction of placental growth by removal of uterine caruncles is associated with low levels of oPL (Falconer *et al.*, 1985). The increase of maternal oPL with arginine infusion is also blunted under these circumstances (Newnham *et al.*, 1986b). There is an increase in maternal oPL following fetal nephrectomy (Brinsmead *et al.*, 1980). Ovine PL binds with high affinity to the human GH receptor, despite the fact that the receptor binding site appears to differ strikingly from that of hGH (Colosi *et al.*, 1989).

Goat placental lactogen

The goat placenta secretes a lactogen with properties very similar to that of the sheep (Currie *et al.*, 1990; Byatt *et al.*, 1992).

(f) Extraction and purification

In the 1960s it was demonstrated that hPL could be prepared from placentas by extraction in aqueous media and purified by standard techniques involving fractional precipitation using salts, DEAE chromatography, polyacrylamide gel electrophoresis and gel filtration. It became clear that high pH should not be used in order to avoid deamidation and aggregation with consequent effects on biological activity. In the method of Hunt *et al.* (1981) frozen placentas were extracted in ammonium bicarbonate at pH 7.8 and the hPL was then adsorbed to DEAE-cellulose from 0.1 M bicarbonate and eluted in 0.4 M bicarbonate. There followed precipitation from ammonium sulphate, gel filtration on Sephadex G-100 and polyacrylamide gel electrophoresis. Monoclinic crystals were produced from solution in 0.1 M phosphate, pH 7.8 and polyethylene glycol-1000.

(g) Chemical structure of hPL

Human placental lactogen is a polypeptide of molecular weight 21 000 with a single chain of 191 amino acids and two intrachain disulphide bonds (Niall *et al.*, 1971, 1973). Studies with recombinant fragments generated by limited plasmin digestion and reduction of hPL and hGH have shown that most of the immunological and biological properties of these hormones are associated with the N-terminal 1–134 amino acids (Russell *et al.*, 1981a). Cleavage of the hPL molecule at residue 86 (tryptophan) completely destroys the immunological and biological activity (Russell *et al.*, 1981b). As in hGH, there is a single tryptophan residue buried within the hydrophobic interior of the molecule. Although there is no evidence that this is hydrogen bonded in the native protein as in hGH, there is a transient bonding in the early stages of thermolysin digestion (Bewley and Li, 1984).

(h) Relation of hPL to other hormones

The amino acid composition of hPL is very similar to that of hGH, the main differences being in the number of methionine, histidine and proline residues. There is an 86% sequence homology, and the disulphide bonds are located in homologous portions of each molecule. Most of the differences are near the N-terminus, and of the observed substitutions only one requires more than a single base change in the triplet codon. The amino acid sequences of hPL and hGH are also similar to that of prolactin (23% sequence homology with hPL, 16% with hGH), though prolactin has 198 amino acids and three intrachain disulphide bonds (Shome and Parlow, 1977).

It appears likely that hPL, hGH and prolactin originated from a shorter primordial peptide of 25–30 amino acids. At an early stage a structural divergence produced two evolutionary lines, one with primarily lactogenic activity, the other with somatotrophic activity. At a later stage individual species showed further divergence within these lines, thus yielding the differences between, for instance, the structure of sheep and human pituitary growth hormone and prolactin. Finally, divergences occurred within species leading to the existence of separate pituitary and placental proteins.

(i) Control mechanisms of hPL

In common with other placental products, there is little evidence for the existence of control mechanisms such as those which exist for pituitary protein hormones. However, there is a large and somewhat confusing literature concerning a number of factors which might influence trophoblastic PL production in both the human and other species.

Prolactin

In rats there is some evidence for an inverse relationship between levels of PL and pituitary prolactin (Nagasawa *et al.*, 1976; Tonkowicz and Voogt, 1984), but similar evidence is not available in the human. Hypophysectomy of the rat can lead to a substantial increase in PL levels (Voogt *et al.*, 1985).

Releasing hormones

Thyrotropin-releasing hormone (TRH), which is a powerful stimulant of pituitary prolactin secretion and is also found in the placenta (Gibbons *et al.*, 1975), has no effect on circulating hPL levels (Hershman *et al.*, 1973). Gonadotrophin-releasing hormone (GnRH) has no effect *in vitro* (Khodr and Siler-Khodr, 1978; Siler-Khodr *et al.*, 1986), though administration of GnRH antagonist has a delayed suppressive effect (Siler-Khodr *et al.*, 1983).

Steroid hormones

In the rat, ovariectomy (M.C. Robertson *et al.*, 1984) produces an increase in PL levels. In the sheep, there is no evidence that endogenous progesterone secretion affects PL secretion (Taylor *et al.*, 1982). Similarly, no effect of oestradiol or progesterone has been shown on secretion of PL by human placental cells *in vitro* (Zeitler *et al.*, 1983).

Carbohydrate and lipid metabolism

Because of the close relationship to growth hormone, several groups have examined the relationship of hPL levels to alterations in carbohydrate and lipid metabolism. Blood levels of hPL decrease following intravenous administration of glucose (Burt *et al.*, 1970; Spellacy *et al.*, 1971; Ajabor and Yen, 1972; Pavlou *et al.*, 1973) (Figure 5.8), though Pavlou and his colleagues showed that the change is small and inconsistent, and might also be attributed to trivial mechanisms such as the osmotic expansion of plasma volume which results from injection of a hypertonic solution.

Increases in blood hPL levels have been shown during insulin-induced hypoglycaemia (Spellacy *et al.*, 1971) prolonged starvation (Kim and Felig, 1971; Brinsmead *et al.*, 1981) and intravenous arginine administration (Prieto *et al.*, 1976), but changes are small when compared with those resulting from the administration of pituitary growth hormone under similar circumstances. In sheep, infusion of high-density lipoproteins (HDL) stimulates PL secretion (Grandis *et al.*, 1989). Equally, a number of studies do not support a significant role of carbohydrate or lipids in the control of hPL secretion. There is no change in hPL levels after oral administration of glucose (Pavlou *et al.*, 1973). In the

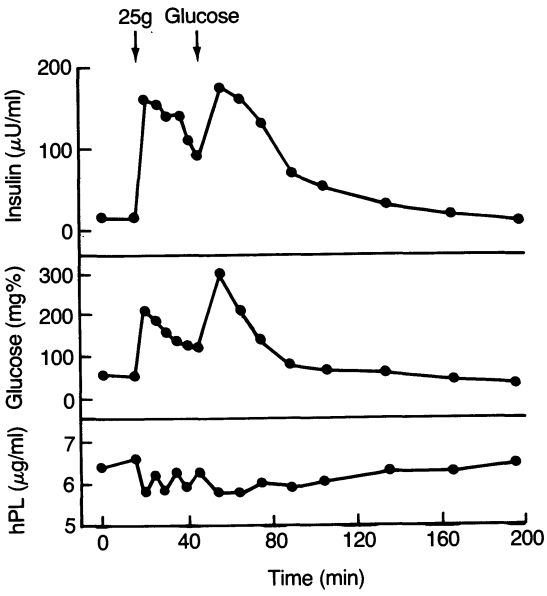

Figure 5.8 Plasma levels of insulin, glucose and hPL in 10 subjects following i.v. injection of glucose.

sheep, maternal hyperglycaemia produces no change in placental lactogen levels in fetus or ewe (Brinsmead *et al.*, 1981). Experimentally induced changes in circulating levels of free fatty acids do not produce any consistent change in hPL concentrations (Morris *et al.*, 1974). The secretion of hPL by dispersed placental cells *in vitro* is not influenced by changes in insulin or glucose concentrations (Zeitler *et al.*, 1983), though contrary results were obtained by Bhaumick *et al.* (1987), who showed that both insulin and insulin-like growth factor (IGF-I) could stimulate hPL production by placental explants.

Other factors

Neither epidermal growth factor (E.A. Wilson *et al.*, 1984b) nor adrenergic β-receptor agonists or antagonists (Shu-Rong *et al.*, 1982) nor corticosteroids (Tatra *et al.*, 1984) have any effect on secretion of hPL by placental cells *in vitro*. Prostaglandin $F_{2\alpha}$ has no direct effect on hPL secretion (Ward *et al.*, 1977). Dopamine reduces hPL production *in vitro* (Macaron *et al.*, 1978), as does transforming growth factor β (Morrish *et al.*, 1991). The latter effect is probably secondary to inhibition of placental differentiation. Ritodrine (Schreyer *et al.*, 1989) and angiotensin (Petit *et al.*, 1989) may enhance hPL secretion *in vivo*.

It has been proposed (Chard, 1981, and Figure 5.9) that the potential for placental production is a function of the total mass of the trophoblast, that the rate of release depends on the concentration of protein in maternal blood in the intervillous space and that this concentration, in turn, depends on the rate of blood flow in the intervillous space.

Thus, hPL will diffuse from the trophoblast cell down a concentration gradient: the faster the rate of blood flow the more rapid is the removal of hPL and hence the steeper the gradient. This hypothesis is compatible with most present evidence and has the important implication that the rate of hPL production and the circulating levels in the mother should be closely related to uteroplacental blood flow. Specific experimental evidence for the concept that the functional mass of the trophoblast is the most important factor in the control of PL secretion comes from studies in the rhesus monkey (Novy *et al.*, 1981). However, studies in the goat have not shown any relationship between short-term variation in uterine blood flow and levels of placental lactogen in the uterine or jugular veins (Hayden *et al.*, 1983). One study (Obiekwe and Chard, 1983a) has shown an apparent sex difference in hPL, higher levels being found in association with a female fetus. This difference is similar to, but less striking than that observed with hCG.

5.4.2 BIOLOGICAL PROPERTIES

(a) Biological effects of hPL

A great variety of biological activities have been proposed for hPL, including growth

promotion, lactogenesis, an effect on carbohydrate and lipid metabolism, stimulation of the corpus luteum, erythropoiesis, inhibition of fibrinolysis and immunosuppression.

Although it is chemically similar to hGH, hPL has only weak somatotrophic activity, variously estimated at 0.1–10% of that of the pituitary protein (Josimovich, 1966; Murakawa and Raben, 1968). However, the growth-promoting activity of hPL may be greater than that of hGH for some fetal tissues (Hill *et al.*, 1985). Sheep fetal fibroblasts have much higher affinity for oPL than oGH (Fowlkes and Freemark, 1992). A placental growth hormone distinct from both hPL and hGH has been described (Hennen *et al.*, 1985) and is the product of the 'silent' hGH-V gene (Frankenne *et al.*, 1987). Maternal levels of hPL are not related to those of insulin-like growth factor (IGF-I) (Kazer *et al.*, 1991).

The extent of the lactogenic activity of hPL has been disputed. Early reports suggested that its potency was some 75% that of sheep prolactin (Josimovich and Brande, 1964). However, later studies showed much lower activity (Friesen, 1965; Florini *et al.*, 1966) (1–4 U/mg in the pigeon crop-sac assay). The potency in the rabbit intraductal assay is greater than that in the pigeon crop-sac assay; a similar discrepancy has been observed with human growth hormone. Studies using intact animals have yielded estimates ranging from 20 to 100% of that of prolactin (Handwerger and Sherwood, 1974). Thus, it is difficult to assign a potency because the apparent lactogenic activity of hPL is highly dependent on the purity of the material examined, on the type of assay and on the animal species used for the assay. Surprisingly, substantial modification of the hPL molecule (reduction and alkylation, oxidation) does not seem to be associated with loss of lactogenic activity (Aloj *et al.*, 1972).

In rodents, hPL has luteotrophic activity and may influence placental progesterone secretion, particularly the interconversion of progesterone and the biologically inactive 20α-dihydroprogesterone (Josimovich and

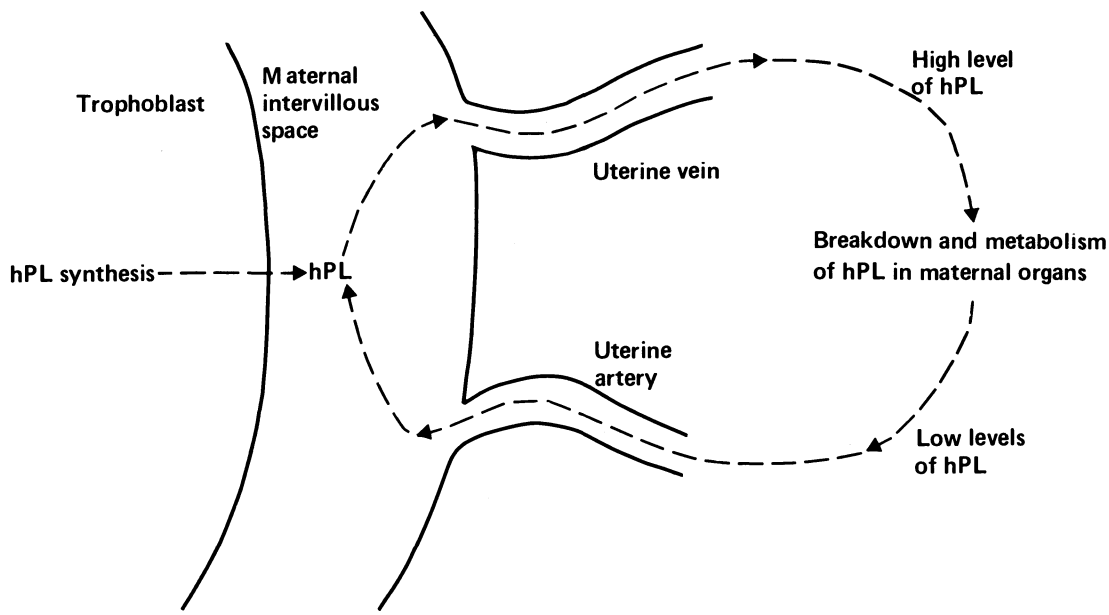

Figure 5.9 How uteroplacental blood flow may control the rate of synthesis of hPL by the trophoblast.

Archer, 1977). Administration of hPL in animals leads to a rise in blood sugar (Burt *et al.*, 1966; Handwerger *et al.*, 1977) and free fatty acids (Riggi *et al.*, 1966); this may be due to increased peripheral resistance to insulin action, though in hypophysectomized rats hPL can cause an increase in insulin release *in vitro* from islet cells (J.M. Martin and Friesen, 1969). Administration of hPL to rhesus monkeys can decrease the hypoglycaemic response to exogenous insulin, without affecting glucose tolerance or the insulin response to glucose (Beck, 1970). PL can modulate insulin production by cultured human fetal pancreas, and it has been suggested that this might contribute to fetal body growth (Swenne *et al.*, 1987).

In the mouse, hPL can stimulate erythropoietin secretion and thus the incorporation of iron into red blood cells (Jepson and Friesen, 1968). In rats, hPL has a direct mitogenic effect on bone marrow *in vitro* (Berczi and Nagy, 1991). Both hPL and hCG can suppress phytohaemagglutinin-induced lymphocyte transformation (Contractor and Davies, 1973; Cerni *et al.*, 1977). The suggestion that either of these compounds plays a significant role in the immune survival of a pregnancy is now largely discredited (reviewed by Chard, 1992).

(b) Biological functions of hPL

It is essential to distinguish between activity and function of hormones. A biological molecule may have a specific effect in an experimental system, but this does not necessarily mean that it is responsible for the same effect in normal physiology. The distinction is especially notable with the biologically active placental proteins: none has been clearly identified as essential for any physiological event, and it has even been suggested that the placental proteins have no biological function but rather are waste products of the general activities of the placenta as an independent organism (Y.B. Gordon and Chard, 1979).

Preparation of the breast for lactation could be an important function of hPL in normal gestation. But there are several other potential candidates for this function, including placental oestrogens and progesterone and maternal pituitary prolactin, all of which are elevated simultaneously. Administration of hPL to non-pregnant subjects in amounts sufficient to yield blood levels similar to those in mid-gestation does not induce lactation (Josimovich *et al.*, 1974). Furthermore, lactation can be induced by breast stimulation even in subjects who have never been pregnant (Anon, 1985).

There are similar reservations on the postulated role of hPL in carbohydrate and lipid metabolism (see Chard, 1992). It is well recognized that pregnancy constitutes a diabetogenic stress, and impairment of carbohydrate tolerance with elevated levels of circulating insulin and free fatty acids are normal findings. It is attractive to speculate that the pregnant woman tends to retain glucose within her circulation where it is available for transport across the placenta to the developing fetus, and that this retention is due to hPL. However, the evidence for this is not strong. Under normal physiological circumstances, there is little relationship between hPL and circulating carbohydrate and lipid levels (see Control mechanisms, section 5.3.4), though a correlation has been shown between hPL and plasma levels of total and free cholesterol, triglycerides and phospholipids measured throughout pregnancy (Desoye *et al.*, 1987). Administration of hPL to non-pregnant subjects leads to impaired carbohydrate tolerance and increased levels of insulin (Beck and Daughaday, 1967; Kalkhoff *et al.*, 1969), but no abnormality in carbohydrate metabolism can be shown in pregnancies in which hPL is absent (Alexander *et al.*, 1982). Furthermore, there are other equally good candidates for an effect on carbohydrate metabolism, such as the pla-

cental steroid hormones (oestrogens and progesterone), which at levels much lower than those in late pregnancy (e.g. in subjects on oral contraceptive agents) can produce all of the changes characteristic of a prediabetic state.

It is attractive to speculate that hPL might be an important growth-promoting factor for the fetus. But this possibility is ruled out by three observations: (i) the somatotrophic activity of hPL is extremely low (1% or less) compared with that of pituitary growth hormone; (ii) the levels of hPL in the fetal circulation are 100-fold less than those in the mother; and (iii) there is no relation between fetal levels of hPL and fetal growth (Houghton *et al.*, 1984).

The lack of any obvious function of PL in human pregnancy is supported by some animal studies. In the sheep, maternal hyperglycaemia produces no change in PL levels in fetus or ewe (Brinsmead *et al.*, 1981). Also in the sheep, neutralization of PL by antibodies does not affect glucose metabolism, or the circulating levels of somatomedins, growth hormone, progesterone or cholesterol (Waters *et al.*, 1985).

(c) Receptors for and mode of action of hPL

Studies on specific tissue receptors for hPL, similar to those available for hGH, are sparse. However, the chorion laeve is believed to contain a lactogen receptor which binds hPL and prolactin (Herington *et al.*, 1980). This receptor is deficient in cases of polyhydramnios (Healy *et al.*, 1985). There is evidence for receptors on the fetal liver in a variety of species (Hill *et al.*, 1988; Freemark *et al.*, 1989). Human PL can bind to the extracellular domain of the prolactin receptor; tight binding is zinc dependent (Lowman *et al.*, 1991). A number of studies have suggested that there may be circulating endogenous binders of placental lactogen (Gusdon *et al.*, 1970, Heal and Gluck, 1980; Southard and Talamantes, 1989).

5.4.3 MEASUREMENTS

(a) Immunoassay for hPL

The majority of studies on hPL have been carried out using radioimmunoassay (see Chard, 1979), though other types of immunological assay have been developed, in particular enzymoimmunoassay (EIA) (Dittmar *et al.*, 1979; MacDonald *et al.*, 1980), fluoroimmunoassay (FIA) (Chard and Sykes, 1979) and turbidimetric assay (Collet-Cassart *et al.*, 1989). The various immunoassays for hPL and the principles underlying them have been reviewed in detail elsewhere (Chard, 1979, 1982, 1983).

(b) Biological and receptor assays for hPL

Though the effects of hPL have been studied in a number of biological systems, these systems have never been used for measurement of the concentration of human PL in biological fluids. Some use has been made of receptor assays (extracts of mammary glands) for the measurement of placental lactogens in other species, e.g. the rat (P.A. Kelly *et al.*, 1976), the cow (Eakle *et al.*, 1982) and the goat (Currie *et al.*, 1977).

5.4.4 CLINICAL APPLICATIONS – DIAGNOSIS

The earlier literature on the diagnostic applications of hPL measurement was reviewed in great detail by Letchworth (1976). Subsequent reviews include those by Chard and Klopper (1982) and Chard (1984). At the present time measurement of circulating hPL is little used as a test of placental function or fetal well-being (Chard, 1987).

(a) Maternal levels of hPL in normal pregnancy

Placental lactogen is detectable in maternal blood soon after implantation; thereafter the levels rise progressively to reach a plateau

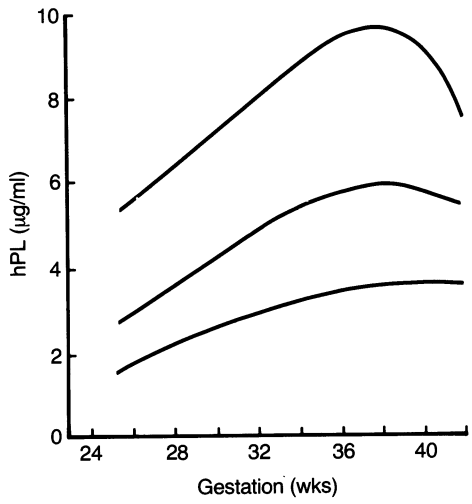

Figure 5.10 The normal range of circulating hPL levels in the mother from weeks 20–42 of gestation (200 subjects). Mean ± 2SD is shown. The distribution is skewed, the variation above the mean being greater than below it.

after the 35th week. This increase follows the sigmoid curve which characterizes all specific placental proteins and which closely parallels the growth of the placenta both in weight and in DNA content. The levels are higher in twin than in singleton pregnancies (Kazer *et al.*, 1991). The levels do not change at the onset of labour and the absolute levels are not related to the time of onset of labour (Gillard *et al.*, 1973).

The normal range of hPL levels in the later part of pregnancy is shown in Figure 5.10.

In common with most other biochemical parameters the variation around the mean shows a skewed distribution; the range of variation is greater above the mean than that below it. Thus, a better approximation to the true mean and standard deviations is obtained after logarithmic transformation of the data. Alternatively, the results are expressed as medians and centiles. In the early first trimester, hPL levels have been used to estimate the stage of gestation (Whittaker *et al.*, 1983), though others have questioned the value of this (Thomson *et al.*, 1988).

(b) Maternal levels of hPL in complications of early pregnancy

Threatened abortion

It is generally agreed that low maternal hPL levels after the 8–10th week are characteristic of threatened abortion with an unsatisfactory outcome and thus provide a guide to prognosis in this condition (Genazzani *et al.*, 1969; Niven *et al.*, 1972; Gartside and Tindall, 1975; Biswas *et al.*, 1980; Hertz and Schultz-Larsen, 1983; Gerhard and Runnebaum, 1984). The low levels would appear to be secondary to death or of damage to the trophoblast, since all placental products are affected in an identical manner (Salem *et al.*, 1984b).

(c) Maternal levels of hPL in complications of late pregnancy

Low birth-weight

Placental lactogen levels are correlated with the weight of the placenta (Sciarra *et al.*, 1968; Saxena *et al.*, 1969; Cramer *et al.*, 1971; Lindberg and Nilsson, 1973; Boyce *et al.*, 1975; Morrison *et al.*, 1980), and of the fetus (Letchworth *et al.*, 1971; Lindberg and Nilsson, 1973; Boyce *et al.*, 1975; Bagga *et al.*, 1990).

The latter relationship is secondary to that between the weight of the placenta and fetus, but there is no doubt that hPL levels are an efficient guide to fetal growth and the prediction of delivery weight in an individual patient (Lindberg and Nilsson, 1973; Hensleigh *et al.*, 1977; MacDonald *et al.*, 1983; Pledger *et al.*, 1984; Westergaard *et al.*, 1985a,b; Laurin *et al.*, 1987), particularly in cases of maternal hypertension (Granat *et al.*, 1977; Sagen *et al.*, 1984). The sensitivity of the test (proportion of positive results in patients with the abnormality) ranges from 29 to 50% depending upon the chosen cut-off point between normal and abnormal (Lilford *et al.*, 1983). Serial levels have better predictive

value than isolated levels (Obiekwe and Chard, 1984). Placental lactogen levels appear to be lower when there is specific evidence of dysmaturity than in those pregnancies where the child is small but normal (Westergaard *et al.*, 1985a). Of the currently available biochemical tests of placental function, hPL is probably marginally superior to all others (Chard *et al.*, 1985).

Low levels of hPL are associated with gross fetal abnormality (Gau and Cadle, 1977). Increased levels occur in twin pregnancies (Garoff and Seppala, 1973; Batra *et al.*, 1978; Briet and Hoorn, 1982; Trapp *et al.*, 1986). The concentration in umbilical cord blood in light-for-dates infants has been reported as elevated (Taketani *et al.*, 1984), but these findings could not be confirmed in another study (Houghton *et al.*, 1984). Elevated levels in cord blood have also been associated with pre-eclampsia (Sprague *et al.*, 1973).

There is evidence that hPL levels in fetal growth retardation only become abnormal after 33 weeks' gestation (Daikoku *et al.*, 1979). Normal levels of hPL are found during mid-trimester in mothers who subsequently deliver a growth-retarded child (Salem *et al.*, 1981).

Pre-eclampsia and hypertension

Spellacy *et al.* (1971) described a 'fetal danger zone' for subjects with hypertension: levels of hPL less than 4 μg/ml after the 30th week indicated a fetal mortality of 24%. Many workers have confirmed the association between low hPL levels and fetal outcome in hypertensive pregnancies (Keller *et al.*, 1971; Lindberg and Nilsson, 1973; Christensen *et al.*, 1974). For example, Kelly *et al.* (1975) have shown a 75% morbidity risk (growth retardation or perinatal asphyxia) for patients developing hypertension after the 28th week and having low hPL levels.

Some confusion in the literature on the precise clinical value of hPL levels in pre-eclampsia can be attributed to three factors. First, the condition itself is very heterogeneous and often not clearly defined. Second, it is very common for authors not to distinguish between pre-eclampsia *per se* and the fetal risk arising from the pre-eclampsia. Thus, in pregnancies in which the only abnormality is hypertension, hPL levels are normal after 36 weeks (Westergaard *et al.*, 1985a,b). Third, studies have looked for low levels of hPL in pre-eclampsia, whereas the best current information suggests that in primigravidas with pre-eclampsia the levels of hPL are elevated (Letchworth and Chard, 1972a; Obiekwe *et al.*, 1984). Placental lactogen levels are generally reduced in cases of antepartum eclampsia (Salem *et al.*, 1983).

Diabetes mellitus

Diabetes mellitus is associated with a large placenta, and it is therefore not surprising that hPL is generally elevated in this condition (Selenkow *et al.*, 1969; M.Cohen *et al.*, 1973; Ursell *et al.*, 1973; Soler *et al.*, 1975; Hertogh *et al.*, 1976; Larinkari *et al.*, 1982; Pledger *et al.*, 1982) (Figure 5.11).

The levels are reduced in cases in which the fetal outcome is unsatisfactory. Thus, hPL levels in a diabetic pregnancy must be interpreted in relation to the elevated range for a 'normal diabetic' pregnancy.

Conflicting results in the literature on hPL levels in diabetes can be attributed to a number of factors, including the definition of the severity of the condition, the failure to appreciate that abnormality should be judged in relation to the general elevation of levels in this condition and the fact that perinatal death may occur due to metabolic dysfunction in the absence of notable placental deficiency. A 'problem' of recent studies is the fact that most diabetic pregnancies are now so well controlled that they are, for all clinical and biochemical purposes, effectively normal (e.g. M.O. Stewart *et al.*, 1989).

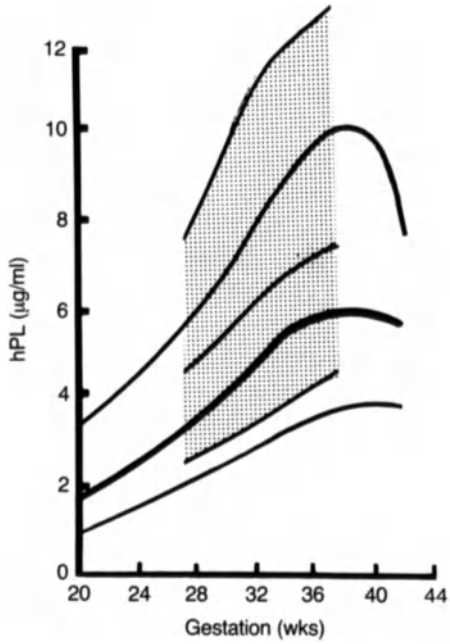

Figure 5.11 hPL levels in pregnancies complicated by diabetes mellitus in which there was no evidence of placental dysfunction (shaded area). Mean ± 2SD of the normal range is also shown.

Rhesus isoimmunization

Placental lactogen levels are generally increased in this condition. The levels are most elevated in cases in which the child is severely affected (Lindberg and Nilsson, 1973; Ward *et al.*, 1974). Prior to the 26th week, a value more than two standard deviations above the normal mean indicates a 90% likelihood that the case will be severe. Similar elevations are seen in amniotic fluid hPL (Niven *et al.*, 1974). The test has been advocated as an additional diagnostic aid at a stage when other parameters, such as liquor bilirubin, may be unhelpful.

Fetal death

Placental lactogen levels often fall before fetal death occurs (Ward *et al.*, 1974). This is compatible with the fact that in many stillbirths the cause is placental insufficiency, rather than a primary defect in the fetus itself. In a controlled study on patients with hypertension, Spellacy *et al.* (1975) showed that action based on hPL levels could lead to a substantial fall in perinatal mortality.

Fetal distress and neonatal asphyxia

Several authors have advocated hPL measurement as a general measure of 'fetal risk' (Letchworth and Chard, 1972b; England *et al.*, 1974; R.P. Edwards *et al.*, 1976; Grudzinskas *et al.*, 1982). There is little doubt that the main factor reflected by maternal hPL levels is fetal size and growth. Consequently, there has been some argument as to whether hPL determinations can predict acute fetal distress in labour, when the latter complication is analysed separately from growth retardation. However, there appears to be an association between abnormal hPL levels and an abnormal result of an oxytocin challenge test (OCT) (Spellacy *et al.*, 1979), and low hPL levels have been shown in association with fetal distress in babies of normal birth weight (Obiekwe and Chard, 1982b).

(d) Other factors which may affect hPL levels

The concentration of hPL tends to fall after 40 weeks' gestation (Lolis *et al.*, 1977). Maternal blood hPL levels are generally lower in smokers than in non-smokers (Moser *et al.*, 1974; Boyce *et al.*, 1975; Spellacy *et al.*, 1977). This is attributable to the associated growth retardation rather than to the smoking itself (J.N. Lee *et al.*, 1980). One study found higher levels of hPL associated with smoking (D'Souza *et al.*, 1988). hPL levels are lower in mothers exposed to continuous aircraft noise (Ando and Hattori, 1977), and elevated levels of hPL have been described in association with low implantation of the placenta (Nicolaides *et al.*, 1982). Low hPL levels may be associated with premature labour (Hercz *et*

al., 1987). Circulating levels of hPL are increased in pregnant renal transplanted women (Klebe *et al.*, 1990). Administration of iron supplements has no effect on hPL levels (Milman *et al.*, 1991).

(e) hPL deficiency

Several authors have described cases of apparent absence of hPL in otherwise uneventful pregnancies (Gaede *et al.*, 1978; Nielsen *et al.*, 1979; Borody and Carlton, 1981; Sideri *et al.*, 1983). Glucose tolerance appears to be normal in these subjects (Alexander *et al.*, 1982). The defects are isolated because other placental products have invariably proved normal in these cases. The deficiency is reflected by a deficiency of mRNA in the trophoblast (Hubert *et al.*, 1983). If both of the hPL genes are deleted there is a total absence of hPL, whereas if only one is deleted there is a relative deficiency, with circulating levels around one-quarter of those normally present (Parks *et al.*, 1985). Complex situations have been described in which there are different deletions on each chromosome (Simon *et al.*, 1986).

(f) Circulating levels of hPL in trophoblast tumours

Trophoblast tumours produce hPL but in smaller amounts than are found in a normal pregnancy (Samaan *et al.*, 1966; Saxena *et al.*, 1968; Goldstein, 1971). The maternal levels appear to be related to tumour differentiation; the greater the degree of malignancy, the lower the hPL value in relation to hCG (Saxena *et al.*, 1968; Goldstein, 1971). In some cases with active disease, hPL may disappear from the circulation while hCG remains detectable (Saxena *et al.*, 1968).

(g) Circulating levels of hPL in non-trophoblast tumours

There have been a number of reports of secretion of hPL by tumours of non-trophoblast origin, including carcinoma of the cervix (Das *et al.*, 1983), the stomach (Shinde *et al.*, 1981; Heyderman *et al.*, 1985) and the oesophagus (Burg-Kurland *et al.*, 1986). Entirely negative results have been reported in large series of cases of ovarian (Monteiro *et al.*, 1983) and breast cancer (Monteiro *et al.*, 1982). High levels have been found, together with hCG, in a case of ovarian dysgerminoma (Kofler and Spona, 1971). Although production of placental proteins (notably SPI and hCG) has been demonstrated in a number of human malignant cell lines *in vitro*, hPL has not been found under these circumstances (Rosen *et al.*, 1980a,b).

5.4.5 CLINICAL APPLICATIONS – THERAPY

(a) hPL as a fertility control vaccine

A high incidence of reproductive loss has been noted in animals immunized with hPL, including rabbits (El-Tomi *et al.*, 1971), rats (Gusdon, 1972) and mice (Yamini *et al.*, 1972). In the last case, it was suggested that the mode of action was an antigen–antibody reaction at the placental site, leading to complement fixation and tissue damage. The possibility of active or passive immunization with hPL to achieve fertility control in humans has not been explored.

REFERENCES

Adejuwon, C.A., Koide, S.S., Mitsudo, S.M. and Segal, S.J. (1980) Apparent chorionic gonadotropin immunoreactivity in human nonplacental tissues, in *Chorionic Gonadotropin*, (ed. S.J. Segal), Plenum Press, New York, pp. 411–419.

Aggarwal, P.B., Farmer, S.W., Papkoff, H. and Stewart, F. (1980) Purification and characterization of donkey chorionic gonadotrophin (abstract). *Journal of Endocrinology*, **85**, 449–455.

Ajabor, I.N. and Yen, S.S.C. (1972) Effect of sustained hyperglycemia on the levels of human chorionic somatomammotropin in mid-pregnancy. *American Journal of Obstetrics and Gynecology*, **112**, 908–911.

Akar, A.H., Wehmann, R.E., Blithe, D.L., Blacker, C. and Nisula, B.C. (1988) A radioimmunoassay for the core fragment of the human chorionic gonadotropin β-subunit. *Journal of Clinical Endocrinology and Metabolism*, **66**, 538–545.

Alexander, I., Anthony, F. and Letchworth, A.T. (1982) Placental protein profile and glucose studies in a normal pregnancy with extremely low levels of human placental lactogen. Case Report. *British Journal of Obstetrics and Gynaecology*, **89**, 241–243.

Alfthan, H., Schroder, U., Fraser, R., Koskimies, A., Halila, H. and Stenman, U.H. (1988) Choriogonadotrophin and its β-subunits separated by hydrophobic-interaction chromatography and quantified in serum during pregnancy by time-resolved immunofluorometric assay. *Clinical Chemistry*, **34**, 1758–1762.

Allen, W.R. (1979) The maternal recognition of pregnancy, in *CIBA Foundation Symposium*, Excerpta Medica, Amsterdam, pp. 323–352.

Aloj, S.M., Edelhoch, H., Handwerger, S. and Sherwood, L.M. (1972) Correlation in the structure and function of human placental lactogen and human growth hormone. II. The effects of disulfide bond modifications on the conformation of human placental lactogen (abstract). *Endocrinology*, **91**, 728–737.

Anderson, B., Milstead, A., Kennedy, G. and Nilson, J.H. (1988) Cyclic AMP phorbol esters interact synergistically to regulate expression of chorionic gonadotrophin genes. *Journal of Biological Chemistry*, **263**, 15578–15584.

Ando, Y. and Hattori, H. (1977) Effects of noise on human placental lactogen (hPL) levels in maternal plasma. *British Journal of Obstetrics and Gynaecology*, **84**, 115–118.

Anon, (1985) Can adoptive mothers breast feed? (abstract). *Lancet*, **2**, 426–427.

Armstrong, E.G., Ehrlich, P.H., Birken, S., Schlatterer, J.P., Siris, E., Hembree, W.C. and Canfield, R.E. (1984) Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, non-pregnant and pregnant individuals. *Journal of Clinical Endocrinology and Metabolism*, **59**, 867–873.

Asch, R.H.J., Fernandez, E.O. and Pauerstein, C.J. (1977) Immunodetection of human chorionic gonadotropin-like substance in human sperm. *Fertility and Sterility*, **28**, 1258–1262.

Aschheim, S. (1930) Early diagnosis of pregnancy, chorionepithelioma and hydatidiform mole by the Aschheim–Zondek test. *American Journal of Obstetrics and Gynecology*, **19**, 335–342.

Aschheim, S. and Zondek, B. (1927a) Das Hormon des Hypophysenvordelappens: Testobjekt zum Nachweis des Hormons. *Klinische Wochenschrift*, **6**, 248–252.

Aschheim, S. and Zondek, B. (1927b) Hypophysenvorderlappenhormon und ovarialhormone im harn von schwangeren. *Klinische Wochenschrift*, **6**, 1322.

Aschheim, S. and Zondek, B. (1928) Die schwangerschaftsdiagnose aus dem harn durch nach weis des hypophysenvorderlappen-hormone. II. Praktische und theoretische ergebnisse aus den harnuntersuchungen. *Klinische Wochenschrift*, **7**, 1453–1457.

Ashitaka, Y., Mochizuki, M. and Tojo, S. (1972) Purification and properties of chorionic gonadotropin from the trophoblastic tissue of hydatidiform mole. *Endocrinology*, **90**, 609–617.

Ayala, A.R., Bustos, H. and Aguilar, R.M.A. (1984) Daily rhythm of serum human chorionic gonadotropin and human chorionic somatomammotropin in normal pregnancy (abstract). *International Journal of Gynecology and Obstetrics*, **22**, 173–176.

Bagga, R., Vasishta, K., Majumdar, S. and Garg, S.K. (1990) Correlation between human placental lactogen levels and glucose metabolism in pregnant women with intrauterine growth retardation. *Australia and New Zealand Journal of Obstetrics and Gynaecology*, **30**, 310–313.

Bagshawe, K.D. (1980) Management of trophoblastic tumours. *Excerpta Medica*, International Congress Series, **512**, 576–578.

Bagshawe, K.D. (1984) Clinical applications of hCG. *Advances in Experimental Biology and Medicine*, **176**, 313–324.

Bahl, O.P. (1969) Human chorionic gonadotrophin. II. Nature of the carbohydrate units. *Journal of Biological Chemistry*, **244**, 575–583.

Bahl, O.P. and Wagh, P.V. (1986) Characterization of glycoproteins: carbohydrate structures of glycoprotein hormones, in *Advances in Experimental Medicine and Biology*, (eds D.S. Dinsha and O.P. Bahl), Plenum Press, New York, pp. 1–51.

Balfour, N.J., Franklyn, J.A., Gurr, J.A. and Sheppard, M.C. (1990) Multiple DNA elements determine basal and thyroid hormone regulated expression of the human glycoprotein α-subunit gene in pituitary cells. *Journal of Molecular Endocrinology*, **4**, 187–190.

Bangham, D.R. and Grab, B. (1964) The second international standard for chorionic gonadotrophin. *Bulletin of the World Health Organization*, **31**, 111–125.

Bangham, D.R. and Storring, P.L. (1982) Standardization of human chorionic gonadotropin, hCG subunits, and pregnancy tests. *Lancet*, **1**, 390.

Barbieri, R.L., Saltzman, D. and Phillipe, M. (1985) Elevated β-human chorionic gonadotropin and testosterone in cord serum of male infants of diabetic mothers. *Journal of Clinical Endocrinology and Metabolism*, **61**, 976–979.

Barbieri, R.L., Saltzman, D.H., Torday, J.S., Randal, R.W., Frigoletto, F.D. and Ryan, K.J. (1986) Elevated concentrations of the beta-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of diabetic mothers. *American Journal of Obstetrics and Gynecology*, **154**, 1039–1043.

Barrera-Saldana, H.A., Seeburg, P.H. and Saunders, G.F. (1983) Two structurally different genes produce the same human placental lactogen hormone. *Journal of Biological Chemistry*, **258**, 3787–3799.

Barsh, G.S., Seeburg, P.H. and Gelinas, R.E. (1983) The human growth hormone gene family: structure and evolution of the chromosomal locus. *Nucleic Acids Research*, **11**, 3939.

Basch, C.V. and Talmantes, F. (1986) *In vitro* kinetics of synthesis and release of mouse placental lactogen. *Endocrinology*, **119**, 1939–1947.

Bates, R.W., Garrison, M.M., Cooper, J.A and Condliffe, P.G. (1968) Further studies on the purification of human thyrotropin. *Endocrinology*, **83**, 721–730.

Batra, S., Sjoberg, N.O. and Aberg, A. (1978) Human placental lactogen, estradiol–17 beta, and progesterone levels in the third trimester and their respective values for detecting twin pregnancy. *American Journal of Obstetrics and Gynecology*, **131**, 69–72.

Batzer, F.R. (1980) Hormonal evaluation of early pregnancy. *Fertility and Sterility*, **34**, 1–13.

Baylin, S.B. and Mendelsohn, G. (1980) Ectopic (inappropriate) hormone production by tumors. Mechanisms involved and the biological and clinical implications. *Endocrine Reviews*, **1**, 45–79.

Beck, P. (1970) Reversal of progesterone-enhanced insulin production by human chorionic somatomammotropin. *Endocrinology*, **87**, 311–315.

Beck, P. and Daughaday, W.H. (1967) Human placental lactogen levels: studies of its acute metabolic effects and disposition in normal man. *Journal of Clinical Investigation*, **1**, 103–110.

Beck, P., Parker, M.L. and Daughaday, W.H. (1965) Radioimmunologic measurement of human placental lactogen in plasma by a double antibody method during normal and diabetic pregnancies. *Journal of Clinical Endocrinology and Metabolism*, **25**, 1457–1462.

Beebe, J.S., Krzesicki, R.F., Norton, S.E., Perini, F., Peters, B.P. and Ruddon, R.W. (1989) Identification and characterization of subpopulations of the free α-subunit that vary in their ability to combine with chorionic gonadotropin-β. *Endocrinology*, **124**, 1613–1624.

Beling, C.G., Cederqvist, L.L. and Fuchs, F. (1976) Demonstration of gonadotropin during the second half of the cycle in women using intrauterine contraception. *American Journal of Obstetrics and Gynecology*, **125**, 855–858.

Belisle, S., Bellabarba, D., Gallo-Payet, N., Lehoux, J.G. and Guevin, V.F. (1986) On the role of luteinizing hormone-releasing hormone in the *in vitro* synthesis of bioactive human chorionic gonadotrophin in human pregnancies. *Canadian Journal of Physiology and Pharmacology*, **54**, 1229–1235.

Bell, J.J., Canfield, R.E. and Sciarra, J.J. (1969) Purification and characterization of human chorionic gonadotropin. *Endocrinology*, **84**, 298–307.

Bellet, D., Arang, J.M., Contesso, G., Gailland, J.M. and Bohuon, C. (1980) Localization of the beta-subunit of human chorionic gonadotrophin on various tumours. *European Journal of Cancer*, **16**, 433–439.

Berczi, I. and Nagy, E. (1991) Placental lactogen is a haemopoietic hormone. *British Journal of Haematology*, **79**, 355–358.

Bewley, T.A. and Li, C.H. (1974) Structural similarities between human pituitary growth hormone, human chorionic somatomammotropin and ovine pituitary growth hormones, in *Lactogenic Hormones, Fetal Nutrition and Lactation*, (ed. B.J. Josimovich), John Wiley & Sons, New York, pp. 19–32.

Bewley, T.A. and Li, C.H. (1984) Conformational comparison of human pituitary growth hormone and human chorionic somatomammotrophin (human placental lactogen) by second-order absorption spectroscopy. *Archives of Biochemistry and Biophysics*, **233**, 219–227.

Bhaumick, B., Dawson, E.P. and Bala, R.M. (1987) The effects of insulin-like growth factor-I and

insulin on placental lactogen production by human term placenta. *Biochemical and Biophysical Research Communications*, **144**, 674–682.

Bidart, J.M., Ozturk, M., Bellett, D.H., Jolivet, M., Grasse-Masse, H., Troalen, F., Bohon, C.J. and Wands, J.R. (1985) Identification of epitopes associated with hCG and beta hCG carboxy terminus by monoclonal antibodies produced against a synthetic peptide. *Journal of Immunology*, **134**, 457–464.

Bidart, J.M., Troalen, F., Salesse, F., Bousfield, G.R., Buhuon, C.J. and Bellet, D.H. (1987) Topographic antigenic determinants recognized by monoclonal antibodies of human choriogonadotropin-β-subunit. *Journal of Biological Chemistry*, **262**, 8551–8556.

Bidart, J.M., Troalen, F., Puisieux, A., Bressac, B., Ozturk, M., Bohuon, C. and Bellet, D. (1988) Recent progress in the immunochemistry of human chorionic gonadotropin, in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 63–72.

Billingsley, S.A. and Wooding, F.B.P. (1990) An immunogold, cryoultrastructural study of sites of synthesis and storage of chorionic gonadotropin and placental lactogen in human syncytiotrophoblast. *Cell and Tissue Research*, **261**, 375–382.

Birken, S., Smith, D.L., Canfield, R.E. and Boime, I. (1977) Partial amino acid sequence of human placental lactogen precursor and its mature hormone form produced by membrane-associated activity. *Biochemical and Biophysical Research Communications*, **74**, 106–116.

Birken, S.E., Armstrong, E.G., Gawinowicz Kolks, M.A., Cole, L.A., Agosto, G.M., Krichevsky, A., Vaitukaitis, J.L. and Canfield, R.E. (1988) Structures of the human chorionic gonadotropin β-subunit fragment from pregnancy urine. *Endocrinology*, **123**, 572–583.

Bischof, P., Martin du Pan, R. and Lauber, K. (1983) Human seminal plasma contains a protein that shares physicochemical, immunochemical and immunosuppressive properties with pregnancy-associated plasma protein-A. *Journal of Clinical Endocrinology*, **56**, 359–362.

Biswas, S., Murrey, M., Buffoe, G., Graves, L., Jelowitz, J. and Dewhurst, J. (1980) Placental lactogen as a reliable index of fetal outcome in threatened abortion during early pregnancy. *Journal of Obstetrics and Gynaecology*, **1**, 75–77.

Blithe, D.L. and Nisula, B.C. (1985) Variations in the oligosaccharides of free and combined α-subunits of human choriogonadotropin in pregnancy. *Endocrinology*, **117**, 2218–2228.

Blithe, D.L., Akar, A.H., Wehmann, R.E. and Nisula, B.C. (1988) Purification of β-core fragment from pregnancy urine and demonstration that its carbohydrate moieties differ from those of native human chorionic gonadotropin-B. *Endocrinology*, **122**, 173–180.

Blobel, G. and Dobberstein, B. (1975) Transfer of proteins across membranes. *Journal of Cell Biology*, **67**, 835–845.

Bo, M. and Boime, I. (1992) Identification of the transcriptionally active genes of the chorionic gonadotropin β gene *in vivo*. *Journal of Biological Chemistry*, **267**, 3179–3184.

Bogart, M.H., Pandian, M.R. and Jones, O.W. (1987) Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. *Prenatal Diagnosis*, **7**, 623–630.

Boime, I. and Boguslawski, S. (1974) The synthesis of human placental lactogen by ribosomes derived from human placenta. *Proceedings of the National Academy of Sciences of the USA*, **74**, 1322–1325.

Boime, I., McWilliams, D., Szcesena, E. and Camel, M. (1976) Synthesis of hPL messenger RNA, a function of gestation. *Journal of Biological Chemistry*, **251**, 820–825.

Bolander, F.F. and Fellows, R.E. (1976) Growth-promoting and lactogenic activities of bovine placental lactogen. *Journal of Endocrinology*, **71**, 173–174.

Bolander, F.F., Ulberg, L.C. and Fellows, R.E. (1976) Circulating placental lactogen levels in dairy and beef cattle. *Endocrinology*, **99**, 1273–1278.

Bolton, R.A., Coulam, C.B. and Ryan, R.J. (1980) Specific binding of human chorionic gonadotropin to human corpora lutea in the menstrual cycle. *Obstetrics and Gynecology*, **56**, 336–338.

Boorstein, W.R., Vamvakopoulos, N.C. and Fiddes, J.C. (1982) Human chorionic gonadotropin β-subunit is encoded by at least eight genes arranged in tandem and inverted pairs. *Nature*, **300**, 419–422.

Boothby, M., Ruddon, R.W., Anderson, C., McWilliams, D. and Boime, I. (1981) A single gonadotropin α-subunit gene in normal tissue and tumour-derived cell lines. *Journal of Biological Chemistry*, **256**, 5121–5127.

Boroditsky, R.S., Reye, F.I., Winter, J.S.D. and Faiman, C.F. (1975) Serum human chorionic

gonadotropin and progesterone patterns in the last trimester of pregnancy: relationship to fetal sex. *American Journal of Obstetrics and Gynecology*, **121**, 238–241.

Borody, I.B. and Carlton, M.A. (1981) Isolated defect in human placental lactogen synthesis in a normal pregnancy. *British Journal of Obstetrics and Gynaecology*, **88**, 447–449.

Bousfield, G.R., Sugino, H. and Ward, D.N. (1985) Demonstration of a COOH-terminal extension on equine lutropin by means of a common acid-labile bond to equine lutropin and equine chorionic gonadotropin. *Journal of Biological Chemistry*, **260**, 9531–9533.

Bousfield, G.R., Liu, W.K., Sugino, H. and Ward, D.N. (1987) Structural studies on equine glycoprotein hormones. Amino acid sequence of equine lutropin β-subunit. *Journal of Biological Chemistry*, **262**, 8610–8620.

Boyce, A., Schwartz, D., Hubert, C., Cedard, I. and Dreyfus, J. (1975) Smoking, human placental lactogen and birth weight. *British Journal of Obstetrics and Gynaecology*, **82**, 864–867.

Bradbury, J.T., Brown, W.E. and Gray, L.A. (1950) Maintenance of the corpus luteum and physiological action of progesterone. *Recent Progress in Hormone Research*, **5**, 151–194.

Braunstein, G.D. (1983) hCG expression in trophoblastic and nontrophoblastic tumors, in *Oncodevelopmental Markers: Biologic, Diagnostic, and Monitoring Aspects*, (ed. W.H. Fishman), Academic Press, New York, pp. 351.

Braunstein, G.D. and Asch, R.H. (1983) Predictive value of analysis of measurements of human chorionic gonadotropin, pregnancy-specific beta-1 glycoprotein, placental lactogen and cystine aminopeptidase for the diagnosis of ectopic pregnancy. *Fertility and Sterility*, **39**, 62–67.

Braunstein, G.D., Vaitukaitis, J.L., Carbone, P.P. and Ross, G.T. (1973) Ectopic production of human chorionic gonadotrophin by neoplasms. *Annals of Internal Medicine*, **78**, 39–43.

Braunstein, G.D., Rasor, J., Adler, D., Danzer, H. and Wade, M.E. (1976) Serum human chorionic gonadotropin levels throughout normal pregnancy. *American Journal of Obstetrics and Gynecology*, **126**, 678–681.

Braunstein, G.D., Karow, W.G., Gentry, W.C., Rasor, T. and Wade, M.M. (1978) First trimester chorionic gonadotropin measurements as an aid in the diagnosis of early pregnancy disorders. *American Journal of Obstetrics and Gynecology*, **143**, 25–32.

Braunstein, G.D., Kamdar, V., Rasor, J., Swaminathan, N. and Wade, M.E. (1979) Widespread distribution of a chorionic gonadotropin-like substance in normal human tissues. *Journal of Clinical Endocrinology and Metabolism*, **49**, 917–922.

Briet, J.W. and Hoorn, R.K.J. (1982) The use of human placental lactogen, oxytocinase and estriol in twin pregnancies with intrauterine growth retardation. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **13**, 7–14.

Brinsmead, M.W., Waters, M.J., Thorburn, G.D., Owens, P.C., Bancroft, B.J. and Tennison, M. (1980) Increase in placental lactogen and somatomedins after fetal nephrectomy. *Journal of Developmental Physiology*, **2**, 205–218.

Brinsmead, M.W., Bancroft, B.J., Thorburn, G.D. and Waters, J. (1981) Fetal and maternal ovine placental lactogen during hyperglycaemia, hypoglycaemia and fasting. *Journal of Endocrinology*, **90**, 337–343.

Broder, L.E., Weintraub, B.D., Rosen, S.W., Cohen, M.H. and Tejada, F. (1977) Placental markers and their subunits as tumor markers in prostatic carcinoma. *Cancer*, **40**, 211–216.

Brody, S. and Carlstrom, G. (1965) Human chorionic gonadotropin pattern in serum and its relation to the sex of the fetus. *Journal of Clinical Endocrinology and Metabolism*, **25**, 792–797.

Burg-Kurland, C.L., Purnell, D.M., Combs, J.W., Valerio, M.G., Harris, C.C. and Trump, B.F. (1986) Immunocytochemical evaluation of primary human esophageal carcinomas and their xenografts for keratin beta-chorionic gonadotropin, placental lactogen, alphafetoprotein, carcinoembryonic antigen, and nonspecific cross-reacting antigen. *Cancer Research*, **46**, 5730–5737.

Burgos, M.H. and Rodriguez, E.M. (1966) Specialized zones in the trophoblast of the human term placenta. *American Journal of Obstetrics and Gynecology*, **96**, 342.

Burnside, J., Buckland, P.R. and Chin, W.W. (1988) Isolation and characterization of the gene encoding the α-subunit of the rat pituitary glycoprotein hormones. *Gene*, **70**, 67–74.

Burt, R.L., Leake, N.H. and Pruitt, A.B. (1966) Observations on the metabolic activity of human placental fraction. *American Journal of Obstetrics and Gynecology*, **95**, 579–585.

Burt, R.L., Leake, N.H. and Rhyne, A.L. (1970) Human placental lactogen and insulin–blood

glucose homeostasis. *Obstetrics and Gynecology*, **36**, 233–237.

Butcher, D.N., Gregory, W.N., Gunter, P.A., Masters, J.R. and Parkinson, M.C. (1985) The biological and clinical significance of hCG-containing cells in seminoma. *British Journal of Cancer*, **51**, 473–478.

Buttle, H.L. and Forsyth, I.A. (1976) Placental lactogen in the cow. *Endocrinology*, **68**, 141–146.

Buttle, H.L., Forsyth, I.A. and Knaggs, G.S. (1972) Plasma prolactin measured by radioimmunoassay and bioassay in pregnant and lactating goats and the occurrence of a placental lactogen. *Journal of Endocrinology*, **53**, 483–491.

Byatt, J.C., Shimomura, K., Duello, T.M. and Bremel, R.D. (1986) Isolation and characterization of multiple forms of bovine placental lactogen from secretory granules of the fetal cotyledon. *Endocrinology*, **119**, 1343–1350.

Byatt, J.C., Warren, W.C., Eppard, P.J., Staten, N.R., Krivi, G.G. and Collier, R.J. (1992) Ruminant placental lactogens: structure and biology. *Journal of Animal Science*, **70**, 2911–2923.

Campbell, K.L., Bagavandoss, P., Byrne, M.D., Jonassen, J.A., Landefeld, T.D., Quasney, M.W., Sanders, M.M. and Midgley, A.R. (1981) Differential processing of the two subunits of human choriogonadotropin by granulosa cells. II. *In vivo* studies. *Endocrinology*, **109**, 1858–1871.

Campbell, R.K., Matzuk, M.M., Canfield, R.E., Boime, I. and Moyle, W.R. (1988) Use of monoclonal antibodies and mutagenesis to study the structure of human chorionic gonadotropin (hCG), in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 123–132.

Canfield, R.E., Morgan, R.J., Kammerman, S., Bell, J.J. and Agosto, G.M. (1971) Studies of human chorionic gonadotropin. *Recent Progress in Hormone Research*, **27**, 121–164.

Canfield, R.E., O'Connor, J.F., Birken, S., Krichevsky, A., Wilcox, A.J., Lustbader, J. and Moyle, W. (1988) hCG immunochemistry as related to clinical applications, in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 55–61.

Canick, J.A., Stevens, L.D., Abell, K.B., Panizza, D.S., Osathanondh, R., Knight, G.J., Palomaki, G.E. and Haddow, J.K.E. (1989) Second trimester maternal serum unconjugated estriol and human chorionic gonadotropin in pregnancies affected with fetal trisomy 18, anencephaly, and open spina bifida. *American Journal of Human Genetics*, **45**, A255.

Carenza, L., Gregorio, R., Mocci, C., Moro, M. and Pala, A. (1980) Ectopic human chorionic gonadotropin: gynaecological tumors and non-malignant conditions. *Gynecological Oncology*, **10**, 32–38.

Catt, K.J., Dufau, M.L. and Tsuruhara, T. (1971) Studies on a radioligand–receptor assay system for luteinizing hormone and chorionic gonadotropin. *Journal of Clinical Endocrinology and Metabolism*, **32**, 860–863.

Caulfield, M.J., Dilkes, M.G., Iles, R.K., Handel, B.T. and Oliver, R.T.D. (1990) Rapid diagnosis of testicular choriocarcinoma by urinary pregnancy tests. *Lancet*, **335**, 1230.

Cerni, C., Tatra, G. and Bohn, H. (1977) Immunosuppression by human placental lactogen (HPL) and the pregnancy-specific beta-1 glycoprotein (SP-1). *Archiv fur Gynakologie*, **223**, 1–7,

Chakravarti, A., Phillips, J.A., Mellits, K.H., Buetow, K.H. and Seeburg, P.H. (1984) Patterns of polymorphism and linkage disequilibrium suggest independent origins of the human growth hormone gene cluster. *Proceedings of the National Academy of Sciences of the USA*, **81**, 6085–6089.

Chambers, W.L. (1949) Adrenal cortical carcinoma in males with excess gonadotropin in urine. *Journal of Clinical Endocrinology*, **9**, 451–456.

Chan, S.Y.W., Chan, P.H., Tang, L.C.H., Ho, P.C. and Tang, G.W.K. (1986) Seminal plasma β-human chorionic gonadotropin (β-hCG): relationships with seminal characteristics and spermatozoal fertilizing capacity. *Andrologia*, **18**, 50–55.

Chang, C.C., Tsong, Y.Y. and Nash, H.A. (1985) Formulation of a potent anti pregnancy vaccine based on the β-subunit of human chorionic gonadotropin (β-hCG). III. Evaluation of various vehicles and adjuvants. *Journal of Reproductive Immunology*, **7**, 163–169.

Chang, D., Sairam, M.R. and Li, C.H. (1973) The primary structure of ovine interstitial cell-stimulating hormone. III. Disulfide bridges of the α-subunit. *Archives of Biochemistry and Biophysics*, **159**, 678–682.

Chang, D., Sairam, M.R and Li, C.H. (1975) The primary structure of ovine interstitial cell-stimulating hormone. IV. Disulfide bridges of the β-subunit. *International Journal of Peptide and Protein Research*, **7**, 487.

Chard, T. (1979) Human placental lactogen, in *Hormones in Blood*, (eds C.H. Gray and V.H.T. James), Academic Press, London, pp. 333–409.

Chard, T. (1981) Synthesis of placental lactogen by human placentae, in *Hormones in Normal and Abnormal Human Tissues*, (eds K. Fotherby and S.B. Pal), Walter de Gruyter, Berlin, p. 4098.

Chard, T. (1982) *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Biomedical Press, Amsterdam.

Chard, T. (1983) Human placental lactogen, in *Hormones in Blood*, (eds C.H. Gray and V.H.T. James), Academic Press, London, pp. 137–145.

Chard, T. (1984) Placental function tests, in *Antenatal and Neonatal Screening*, (ed. N.J. Wald), Oxford University Press, Oxford, pp. 510–522.

Chard, T. (1987) What is happening to placental function tests? *Annals of Clinical Biochemistry*, **24**, 435–439.

Chard, T. (1991) Frequency of implantation and early pregnancy loss in natural cycles. *Bailliere's Clinical Obstetrics and Gynaecology*, **5**, 179–189.

Chard, T. (1992) Placental hormones and metabolism, in *Medicine of the Fetus and Mother*, (eds L.A. Reece, J.C. Hobbins, M.J. Mahoney and R.H. Petrie), J.B. Lippincott Philadelphia, pp. 88–96.

Chard, T. and Grudzinskas, J.G. (1992) Placental proteins and steroids and the immune relationship between mother and fetus, in *Immunological Obstetrics*, (eds C.B. Coulam, W. Page Faulk and J.A. McIntyre), W.W. Norton, New York, pp. 282–289.

Chard, T. and Klopper, A. (1982) *Placental Function Tests*, Springer-Verlag, Berlin.

Chard, T. and Sykes, A. (1979) Fluoroimmunoassay for human choriomammotropin. *Clinical Chemistry*, **25**, 973–975.

Chard, T., Sturdee, J., Cockrill, B. and Obiekwe, B.C. (1985) Which is the best placental function test? A comparison of placental lactogen and unconjugated oestriol in the prediction of intrauterine growth retardation. *European Journal of Obstetrics, Gynaecology and Reproductive Biology*, **19**, 13–17.

Chard, T., Craig, P.H., Menabawey, M. and Lee, C. (1986) Alpha interferon in human pregnancy. *British Journal of Obstetrics and Gynaecology*, **93**, 1145–1149.

Chartier, M.M., Roger, M., Barratt, J. and Michelson, B. (1979) Measurement of plasma hCG and beta-hCG in the late luteal phase: evidence of the occurrence of spontaneous menstrual abortions in infertile women. *Fertility and Sterility*, **31**, 134–137.

Chen, F. and Puett, D. (1992) A single amino acid residue replacement in the β-subunit of human chorionic gonadotrophin results in the loss of biological activity. *Journal of Molecular Endocrinology*, **8**, 87–89.

Chen, H.C., Hodgen, G.D., Matsuwra, S., Lin, L.J., Gross, L., Reichet, L.E., Birken, S., Canfield, R.E. and Ross, G.T. (1976) Evidence for a gonadotropin from non-pregnant subjects that has physical, immunological and biological similarities to human chorionic gonadotropin. *Proceedings of the National Academy of Sciences of the USA*, **73**, 2885–2889.

Chen, H.C., Matsuura, S., Hodgen, G.D. and Ross, G.T. (1978) A human chorionic gonadotropin-like substance from human luteinizing hormone, in *Novel Aspects of Reproductive Physiology*, (eds G.H. Spitman and J.W. Wilks), Spectrum Publishers, New York, pp. 289–308.

Chen, H.C., Shimohigashi, Y., Dufau, M.C. and Catt, K.J. (1982) Characterization and biological properties of chemically deglycosylated human chorionic gonadotropins. *Journal of Biological Chemistry*, **257**, 14446–14452.

Chiang, M.H. and Nicoll, C.S. (1992) Human placental lactogen inhibits growth without changing serum levels of IGF-1 in rats: an apparent specific action of the hormone. *Acta Endocrinologica*, **127**, 146–151.

Chin, W.W. (1988) Hormonal regulation of thyrotropin and gonadotropin gene expression. *Clinical Research*, **36**, 484–493.

Chin, W.W. and Gharib, S.D. (1986) Organization and expression of gonadotropin genes. *Advances in Medicine and Biology*, **205**, 345–365.

Chou, J.Y., Hugumin, P.E. and Mano, T. (1983) Control of placental protein production by retinoic acid in cultured placental cells. *In vitro*, **19**, 571–575.

Christakos, S. and Bahl, O.P. (1979) Pregnant mare serum gonadotropin: purification and physico-chemical, biological and immunological characteristics. *Journal of Biological Chemistry*, **254**, 4253–4261.

Christensen, A., Frayshov, O. and Fylling, P. (1974) Hormone and enzyme assays in pregnancies. *Acta Endocrinologica*, **77**, 344–355.

Cohen, H., Grumbach, M.M. and Kaplan, S.L. (1964) Preparation of human chorionic 'growth hormone–prolactin'. *Proceedings of the Society for Experimental Biology and Medicine*, **117**, 438–441.

Cohen, M., Haour, F., Dumont, M. and Bertrand, J. (1973) Prognostic value of human chorionic somatomammotropin plasma levels in diabetic patients. *American Journal of Obstetrics and Gynecology*, **115**, 202–210.

Cole, H.H. and Hart, G.H. (1930) The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturation of the immature rat. *American Journal of Physiology*, **93**, 57–68.

Cole, L.A. (1987) Distribution of *O*-linked sugar units on hCG and its free α-subunit. *Molecular and Cellular Biology*, **50**, 45–57.

Cole, L.A. and Birken, S. (1988) Origin and occurrence of human chorionic gonadotropin β-subunit core fragment. *Molecular Endocrinology*, **2**, 825–830.

Cole, L.A. and Nam, J.H. (1989) Urinary gonadotropin fragments (UGF) measurements in the diagnosis and management of ovarian cancer. *Yale Journal of Biology and Medicine*, **62**, 367–378.

Cole, L.A., Kroll, T.G., Ruddon, R.W. and Hussa, R.O. (1984a) Differential occurrence of free beta and free alpha subunits of human chorionic gonadotrophin (hCG) in pregnancy sera. *Journal of Clinical Endocrinology and Metabolism*, **58**, 1200–1202.

Cole, L.A., Perini, F., Birken, S. and Ruddon, R.W. (1984b) An oligosaccharide of the *O*-linked type distinguishes the free from the combined form of hCG alpha subunit. *Biochemical and Biophysical Research Communications*, **122**, 1260–1267.

Cole, L.A., Wang, Y., Elliot, M., Latiff, M., Chambers, J.T., Chambers, S.K. and Schwartz, P.E. (1988) Urinary human chorionic gonadotrophin free β-subunit and β-core fragment a new marker of gynaecological cancers. *Cancer Research*, **48**, 1356–1360.

Cole, L.A., Kardana, A. and Birken, S. (1990) The isomers, subunits and fragments of hCG. *Serono Symposium*, **65**, 59–79.

Cole, L.A., Kardana, A., Park, S.Y. and Braunstein, G.D. (1993) The deactivation of hCG by nicking and dissociation. *Journal of Clinical Endocrinology and Metabolism*, **76**, 704–710.

Collet-Cassart, D., Limet, J.N., Van Krieken, L. and De Hertogh, R. (1989) Turbidimetric latex immunoassay of placental lactogen on microtiter plates. *Clinical Chemistry*, **35**, 141–143.

Colosi, P., Thordarson, G., Hellmiss, R., Singh, K., Forsyth, I.A., Gluckman, P. and Wood, W.I. (1989) Cloning and expression of ovine placental lactogen. *Molecular Endocrinology*, **3**, 1462–1469.

Combarnous, Y. (1981) Physico-chemical properties of pregnancy mare serum gonadotropin. *Biochimica et Biophysica Acta*, **667**, 267–276.

Contractor, S.F. and Davies, H. (1973) Effect of human chorionic somatomammotropin and human chorionic gonadotrophin on phytohaemagglutinin-induced lymphocyte transformation. *Nature New Biology*, **243**, 284–285.

Corless, C.L., Matzuk, M.M., Ramabhadran, T.V., Krichevsky, A. and Boime, I. (1987) Gonadotropin beta subunits determine the rate of assembly and the oligosaccharide processing of hormone dimer in transfected cells. *Journal of Cell Biology*, **104**, 1173–1181.

Cornell, J.S. and Pierce, J.G. (1974) Studies on the disulphide bonds of glycoprotein hormones, locations in the alpha chain based on partial reductions and formation of the 14-C-labelled S-carboxymethyl derivatives. *Journal of Biological Chemistry*, **249**, 4166–4174.

Cox, G.S., Weintraub, B.D., Rosen, S.W. and Maxwell, E.S. (1976) Properties of biologically active messenger RNA from human placenta. *Journal of Biological Chemistry*, **251**, 1723–1728.

Cramer, D.W., Beck, B. and Makowski, E.L. (1971) Correlation of gestational age with maternal human chorionic somatomammotropin and maternal and fetal growth hormone plasma concentrations during labor. *American Journal of Obstetrics and Gynecology*, **109**, 649–655.

Crosignani, P.G., Nencioni, T. and Brambati, B. (1972) Concentration of chorionic gonadotrophin and chorionic somatomammotrophin in maternal serum, amniotic fluid and cord blood serum at term. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **79**, 122–126.

Csapo, A.I. and Pulkkinen, M. (1978) Indispensability of the human corpus luteum in the maintenance of early pregnancy: luteectomy evidence. *Obstetrical and Gynaecological Survey*, **33**, 69–81.

Currie, W.B., Kelly, P.A., Friesen, H.G. and Thorburn, G.D. (1977) Caprine placental lactogen: levels of prolactin-like growth hormone-like activities in the circulation of pregnant goats determined by radioreceptor assays. *Journal of Endocrinology*, **73**, 215–226.

Currie, W.B., Card, C.E., Michel, F.J. and Ignotz, G. (1990) Purification and partial characterization, and development of a specific radio-

immunoassay for goat placental lactogen. *Journal of Reproduction and Fertility*, **90**, 25–36.

D'Souza, S.W., Lieberman, B., Cadman, J. and Richards, B. (1988) Maternal serum placental lactogen and α-fetoprotein in smokers: relationship to placental weight and birth weight. *Journal of Obstetrics and Gynaecology*, **9**, 94–99.

Daikoku, N.H., Tyson, J.E., Graf, C., Scott, R., Smith, B., Johnson, J.W.C. and King, T.M. (1979) The relative significance of human placental lactogen in the diagnosis of retarded fetal growth. *American Journal of Obstetrics and Gynecology*, **135**, 516–520.

Damm, J.B.L., Boshol, H., Hard, K., Kamerling, J.P., Van Dedem, G.W.K. and Vliegenthart, J.F.G. (1988) The β-subunit of human chorionic gonadotropin contains N-glycosidic trisialo tri and tri-antennary carbohydrate chains. *Glycoconjugate Journal*, **5**, 221–233.

Das, S., Mukherjee, K., Battacharya, S. and Chowdhury, J.R. (1983) Ectopic production of placental hormones (human chorionic gonadotropin and human placental lactogen) in carcinoma of the uterine cervix. *Cancer*, **51**, 1854–1857.

Day, J.R., Ogren, L. and Talamantes, F. (1986) The effect of hypophysectomy on serum placental lactogen and progesterone in the mouse. *Endocrinology*, **119**, 259–269.

Deb, S., Faria, T.N., Roby, K.F., Larsen, D., Kwok, S.C., Talamantes, F. and Soares, M.J. (1991) Identification and characterization of a new member of the prolactin family, placental lactogen-I variant. *Journal of Biological Chemistry*, **266**, 1605–1610.

Desoye, G., Schweditsch, M.O., Pfeiffer, K.P., Sechner, R. and Kostner, G.M. (1987) Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum. *Journal of Clinical Endocrinology and Metabolism*, **64**, 704–712.

Dias, J.A. (1992) Recent progress in structure–function and molecular analyses of the pituitary/placental glycoprotein hormone receptors (abstract). *Biochimica et Biophysica Acta*, **1135**, 278–294.

Diczfalusy, E., Hogberg, B. and Westman, A. (1950) Use of the ventral prostate in the assay of human chorionic gonadotropin. *Acta Endocrinologica*, **5**, 226–234.

Dittmar, D., Monji, N., Cid, A., Malkus, H. and Castro, A. (1979) Enzyme-linked immunosorbent choriomammotropin assay. *Clinical Chemistry*, **25**, 227–229.

Duckworth, M.L., Kirk, K.L. and Friesen, H.G. (1986) Isolation and identification of a cDNA clone of rat placental lactogen. II. *Journal of Biological Chemistry*, **261**, 10871–10878.

Duello, T.M., Byatt, J.C. and Bremel, R.D. (1986) Immunohistochemical localization of placental lactogen in binucleate cells of bovine placentomes. *Endocrinology*, **119**, 1351–1355.

Dufau, M.L., Mendelson, C. and Catt, K.J. (1974) A highly sensitive bioassay for luteinizing hormone and chorionic gonadotropin: testosterone production by dispersed Leydig cells. *Journal of Clinical Endocrinology and Metabolism*, **39**, 610–613.

Eakle, K.A., Arima, Y., Swanson, P., Grimek, H. and Bremel, R.D. (1982) A 32, 000 molecular weight protein from bovine placenta with placental lactogen-like activity in radioreceptor assays. *Endocrinology*, **110**, 1758–1765.

Edmonds, D.K., Miller, K.S., Miller, J.R., Williamson, E. and Wood, P.J. (1982) Early embryonic mortality in women. *Fertility and Sterility*, **39**, 447–453.

Edwards, R.G. and Steptoe, P.C. (1983) Current status of *in vitro* fertilisation and implantation of human embryos. *Lancet*, **2**, 112651269.

Edwards, R.P., Diver, M.J., Davis, J.C. and Hipkin, L. (1976) Plasma oestriol and human placental lactogen measurements in patients with high risk pregnancies. *British Journal of Obstetrics and Gynaecology*, **83**, 1229237.

Ehrlich, P.H., Moyle, W.R., Moustafa, Z.A. and Canfield, R.E. (1982) Mixing two monoclonal antibodies yields enhanced affinity for antigen. *Journal of Immunology*, **128**, 1709–2713.

El-Deiry, S., Kaetzel, D., Kennedy, G., Nilson, J. and Puett, D. (1989) Site-directed mutagenesis of the human chorionic gonadotropin β-subunit: bioactivity of a heterologous hormone, bovine α-subunit 1(122145)B. *Molecular Endocrinology*, **3**, 115231528.

El-Tomi, A.E.F., Crystle, C.D. and Stevens, V.C. (1971) Effects of immunization with human placental lactogen on reproduction in female rabbits. *American Journal of Obstetrics and Gynecology*, **109**, 17480.

Elkind-Hirsch, K.E., Reynolds, M.V. and Goldzieher, J.W. (1989) Comparison of immunoreactive gonadotrophin releasing hormone on human chorionic gonadotrophin in term placentas from normal women and those with insulin-

dependent and gestational diabetes. *American Journal of Obstetrics and Gynecology*, **160**, 17-78.

Endo, Y., Yamashita, K., Tachibana, Y., Tojo, S. and Kobata, A. (1979) Structures of the asparagine-linked sugar chains of human chorionic gonadotropin. *Journal of Biochemistry (Tokyo)*, **85**, 669–679.

England, P., Lorrimer, D., Fergusson, J.C., Moffatt, A.M. and Kelly, A.M. (1974) Human placental lactogen: the watchdog of fetal distress. *Lancet*, **1**, 5–6.

Falconer, J., Owens, J.A., Allotta, E. and Robinson, J.S. (1985) Effect of restriction of placental growth on the concentrations of insulin, glucose and placental lactogen in the plasma of sheep. *Journal of Endocrinology*, **106**, 7–11.

Fenstermaker, R.A., Milstead, A., Virgin, J.B., Miller, W.L. and Nilson, J.H. (1989) The transcriptional response of the human chorionic gonadotropin β-subunit gene to cAMP is cycloheximide sensitive and is mediated by cis-acting sequences different from that found in the α-subunit gene. *Molecular Endocrinology*, **3**, 11070–1076.

Fiddes, J.C. and Goodman, H.M. (1979) Isolation, cloning and sequence analysis of the cDNA for the α-subunit of human chorionic gonadotrophin. *Nature*, **281**, 351–356.

Fiddes, J.C. and Goodman, H.M. (1980) The cDNA for the β-subunit of human chorionic gonadotropin suggests evolution of a gene by readthrough into the 3'-untranslated region. *Nature*, **386**, 648–687.

Fiddes, J.C. and Talmadge, K. (1984) Structure, expression and evolution of the genes for the human glycoprotein hormones. *Recent Progress in Hormone Research*, **40**, 43–53.

Fielder, P.J. and Talamantes, F. (1987) The lipolytic effects of mouse placental lactogen II, mouse prolactin, and mouse growth hormone on adipose tissue from virgin and pregnant mice. *Endocrinology*, **121**, 493–497.

Fielder, P.J., Robleto, D.O., Ogren, L. and Talamantes, F. (1989) Ethanol consumption during pregnancy in mice: effects on hormone concentrations. *American Journal of Physiology*, **257**, E561–E566.

Filstein, M.R., Culliman, J.A. and Strauss, J.F. (1983) Aberrant results in serum β-human chorionic gonadotropin assays. An infrequent but vexing problem. *Fertility and Sterility*, **39**, 714–716.

Fishel, S.B., Edwards, R.G. and Evans, C.J. (1984) Human chorionic gonadotropin secreted by preimplantation embryos cultured *in vitro*. *Science*, **233**, 816–818.

Flint, A.P.F., Lamming, E.G. and Stewart, H.J. (1988) A role for interferons in the maternal recognition of pregnancy. *Molecular and Cellular Endocrinology*, **58**, 109–111.

Florini, J.R., Tonelli, G., Breuer, C.B., Coppola, J., Ringler, I. and Bell, P.H. (1966) Characterization and biological effects of purified placental protein (human). *Endocrinology*, **79**, 692–708.

Forsyth, I.A. and Bloomfield, G. (1986) Variation among species in the endocrine control of mammary growth and function: the roles of prolactin and growth hormone, and placental lactogen. *Journal of Dairy Science*, **69**, 886–903.

Fowlkes, J. and Freemark, M. (1992) Binding of placental lactogen and growth hormone to fetal sheep fibroblasts. *Pediatric Research*, **32**, 200–203.

Frankenne, F., Rentier-Delrue, F., Scippo, M.L., Martial, J. and Hennen, G. (1987) Expression of the growth hormone variant gene in human placenta. *Journal of Clinical Endocrinology and Metabolism*, **64**, 635–637.

Freemark, M. and Handwerger, S. (1986) The glycogenic effects of placental lactogen and growth hormone in ovine fetal liver are mediated through binding to specific fetal oPL receptors. *Endocrinology*, **118**, 613–618.

Freemark, M., Comer, M., Mularoni, T., D'Ercole, A.J., Grandis, A. and Kodak, L. (1989) Nutritional regulation of the placental lactogen receptor in fetal liver: implications for fetal metabolism and growth. *Endocrinology*, **125**, 1504–1512.

Freemark, M., Keen, A., Fowlkes, J., Mularoni, T., Comer, M., Grandis, A. and Kodak, L. (1992) The placental lactogen receptor in maternal and fetal sheep liver: regulation by glucose and role in the pathogenesis of fasting during pregnancy. *Endocrinology*, **130**, 1063–1070.

Friedman, M.H. and Lapham, M.E. (1931) A simple rapid procedure for the laboratory diagnosis of early pregnancies. *American Journal of Obstetrics and Gynecology*, **21**, 405–410.

Friesen, H. (1965) Purification of placental factor with immunological and chemical similarity to human growth hormone. *Endocrinology*, **76**, 369–381.

Fujiki, Y., Rathnam, P. and Saxena, B.B. (1980) Studies on the disulfide bonds in human pitu-

itary follicle-stimulating hormone. *Biochimica et Biophysica Acta*, **624**, 428–485.

Gaede, P., Trolle, D. and Pedersen, H. (1978) Extremely low placental lactogen hormone values in an otherwise uneventful pregnancy. *Acta Obstetrica et Gynecologica Scandinavica*, **57**, 203.

Garoff, L. and Seppala, M. (1973) Alpha feto-protein and human placental lactogen levels in maternal serum in multiple pregnancies. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 695–700.

Gartside, M.W. and Tindall, V.R. (1975) The prognostic value of human placental lactogen (HPL) levels in threatened abortion. *British Journal of Obstetrics and Gynaecology*, **82**, 303–309.

Gaspard, V.J., Reuter, A.M., Deville, J.L., Vrindts-Gevaert, Y., Bagshawe, K.D. and Franchimont, P. (1980) Serum concentrations of human chorionic gonadotrophin and its α and β subunits. 2. Trophoblastic tumours. *Clinical Endocrinology*, **13**, 319–329.

Gau, G. and Cadle, G. (1977) Placental lactogen levels associated with gross fetal abnormality. *Obstetrics and Gynecology*, **49**, 243–244.

Gau, G. and Chard, T. (1975) The distribution of placental lactogen in the human term placenta. *British Journal of Obstetrics and Gynaecology*, **82**, 790–793.

Genazzani, A.R., Aubert, M.L., Casoli, M., Fioretti, P. and Felber, J.P. (1969) Use of human-placental-lactogen radioimmunoassay to predict outcome in cases of threatened abortion. *Lancet*, **2**, 1385–1387.

George, D.L., Phillips, J.A., Francke, U. and Seeburg, P.H. (1981) The genes for growth hormone and chorionic somatomammotropin are on the long arm of chromosome 17 in region q21-qter. *Human Genetics*, **57**, 138–141.

Gerhard, I. and Runnebaum, B. (1984) Predictive value of hormone determinations in the first half of pregnancy. *European Journal of Gynecology and Reproductive Biology*, **17**, 1–7.

Gibbons, J.M., Mitnick, M. and Chieffo, V. (1975) *in vitro* biosynthesis of TSH- and LH-releasing factors by the human placenta. *American Journal of Obstetrics and Gynecology*, **121**, 127–133.

Gillard, M., Letchworth, A.T. and Chard, T. (1973) Human placental lactogen levels in relation to the initiation and maintenance of labour. *Obstetrics and Gynecology*, **41**, 774–776.

Goldstein, D.P. (1971) Serum placental lactogen activity in patients with molar pregnancy and trophoblastic tumors – a reliable index of malignancy. *American Journal of Obstetrics and Gynecology*, **110**, 583–587.

Gordon, D.F., Wood, W.M. and Ridgeway, E.C. (1988) Organization and nucleotide sequence of the mouse α-subunit of the pituitary hormones. *DNA*, **7**, 679–690.

Gordon, Y.B and Chard, T. (1979) The specific proteins of the human placenta: some new hypotheses, in *Placental Proteins*, (eds A. Klopper and T. Chard), Springer-Verlag, Berlin, pp. 1–21.

Got, R. and Bourrillon, R. (1960) Nouvelles données physiques sur le gonadotrophine choriale humaine. *Biochimica et Biophysica Acta*, **39**, 241–247.

Goverde, B.C., Veenkamp, F.J.N. and Homan, J.D.H. (1968) Studies on human chorionic gonadotrophin. II. Chemical composition and its relation to biological activity. *Acta Endocrinologica*, **59**, 110–119.

Graham, M.Y., Otani, T., Boime, I., Olson, M.V., Carle, G.F. and Chaplin, D.D. (1987) Cosmid mapping of the human chorionic gonadotropin β-subunit genes by field-inversion gel electrophoresis. *Nucleic Acids Research*, **15**, 4437–4448.

Granat, M., Sharf, M., Diengott, D., Spindel, A., Kahana, L. and Elrad, H. (1977) Further investigation of the predictive value of human placental lactogen in high-risk pregnancies. *American Journal of Obstetrics and Gynecology*, **129**, 647–654.

Grandis, A., Jorgensen, V., Kodak, L., Quarfordt, S. and Handwerger, S. (1989) High-density lipoproteins (HDL) stimulate placental lactogen secretion in pregnant ewes: further evidence for a role of HDL in placental lactogen secretion during pregnancy. *Journal of Endocrinology*, **120**, 423–427.

Gray, C.J. (1988) Glycoprotein gonadotropins. Structure and synthesis. *Acta Endocrinologica (Copenhagen)*, **288**, 20–27.

Green, E.D., Boime, I. and Baenziger, J.U. (1986) Differential processing of Asn-linked oligosaccharides on pituitary glycoprotein hormones: implications for biologic function. *Molecular and Cellular Biology*, **72**, 81–100.

Griffin, J. and Odell, W.D. (1987) Two monoclonal Ab-sandwich-type assays of human lutropin with no cross reaction with choriogonadotropin. *Clinical Chemistry*, **33**, 1603–1607.

Grudzinskas, J.G., Menabawey, M., Wiley, B.A., Teisner, B. and Chard, T. (1979) The relation

between the concentration of placental specific proteins in retroplacental and peripheral blood. *British Journal of Obstetrics and Gynaecology*, **86**, 891–893.

Grudzinskas, J.G., Gordon, Y.B., Wadsworth, J., Menabawey, M. and Chard, T. (1982) Is placental function testing worthwhile? *Australia and New Zealand Journal of Obstetrics and Gynaecology*, **21**, 103–105.

Grumbach, M. and Kaplan, S.L. (1964) On the placental origin and purification of chorionic 'growth hormone-prolactin' and its immunoassay in pregnancy. *Annals of the New York Academy of Science*, **27**, 167–188.

Gusdon, J.P. (1972) An immunologic method of pregnancy destruction and contraception. *American Journal of Obstetrics and Gynecology*, **112**, 472.

Gusdon, J.P. and Sain, L.E. (1981) Uterine and peripheral blood concentrations of human chorionic gonadotropin and human placental lactogen. *American Journal of Obstetrics and Gynecology*, **139**, 705–707.

Gusdon, J.P., Leake, N.H. and Burt, R.L. (1970) Naturally occurring antibody to placental protein in human pregnancy. *American Journal of Obstetrics and Gynecology*, **108**, 1056–1062.

Hammond, C.B., Clarke-Pearson, D.L. and Soper, J.T. (1984) Management of patients with gestational trophoblastic neoplasia: experience of the Southeastern Regional Center. *Advances in Medicine and Biology*, **176**, 369–381.

Handwerger, S. and Sherwood, I.M. (1974) *Methods of Hormone Radioimmunoassay*, in (eds B.M. Jaffe and H.F. Behrman), Academic Press, New York, pp. 427–436.

Handwerger, S., Crenshaw, C., Maurer, W.F., Barrett, J., Hurley, T.W., Golander, A. and Fellows, R.E. (1977) Studies on ovine placental lactogen secretion by homologous radioimmunoassay. *Journal of Endocrinology*, **72**, 27–34.

Handwerger, S., Grandis, A., Barry, S. and Crenshaw, M.C. (1981) Stimulation by ornithine of ovine placental lactogen secretion. *Journal of Endocrinology*, **88**, 283–288.

Handwerger, S., Wilson, S.P., Tyrey, L. and Conn, P.M. (1987) Biochemical evidence that human placental lactogen and human chorionic gonadotropin are not stored in cytoplasmic secretion granules. *Biology of Reproduction*, **37**, 28–32.

Hanson, F.W., Powell, J.E. and Stevens, V.C. (1971) Effects of hCG and human pituitary LH on steroid secretion and functional life of the human corpus luteum. *Journal of Clinical Endocrinology*, **32**, 211–215.

Harper, M.E., Barrera-Saldana, H.A. and Saunders, G.F. (1982) Chromosomal localization of the human placental growth hormone gene cluster to 17Q22–24. *American Journal of Obstetrics and Gynecology*, **34**, 227–234.

Harris, D.C., Machin, K.J., Evin, G.M., Morgan, F.J. and Isaacs, N.W. (1989) Preliminary X-ray diffraction analysis of human chorionic gonadotropin. *Journal of Biological Chemistry*, **264**, 6705–6706.

Hartree, A.S., Shownkeen, R.C., Stevens, V.C., Matsuura, S., Ohashi, M. and Chen, H.C. (1983) Studies of the human chorionic gonadotrophin-like substance of human pituitary glands and its significance. *Journal of Endocrinology*, **96**, 115–126.

Hayden, T.J., Buttle, H.L., Rees, P.L., Smith, S.V. and Forsyth, I.A. (1983) Simultaneous determinations of uterine blood flow and plasma concentrations of placental lactogen in late-pregnant goats. *Journal of Reproduction and Fertility*, **69**, 503–510.

Heal, A.V. and Gluck, J. (1980) Qualitative detection of human placental lactogen-binding substance by radioassay. *American Journal of Obstetrics and Gynecology*, **137**, 975–977.

Healy, D., Herington, A.C. and O'Herlihy, C. (1985) Chronic polyhydramnios is a syndrome with a lactogen receptor defect in the chorion laeve. *British Journal of Obstetrics and Gynaecology*, **92**, 461–467.

Hearn, J.P. (1976) Immunization against pregnancy. *Proceedings of the Royal Society of London*, **195**, 149–160.

Hearn, J.P., Short, R.V. and Lunan, S.F. (1976) The effect of immunizing marmoset monkeys against the β-subunit of hCG, in *Physiological Effects of Immunity against Reproductive Hormones*, (eds R.G. Edwards and M.H. Johnson), Cambridge University Press, Cambridge, pp. 2298–242.

van Hell, H., Matthijsen, R. and Homan, J.D.H. (1968) Studies on human chorionic gonadotrophin. I. Purification and some physicochemical properties. *Acta Endocrinologica*, **59**, 89–104.

Hennen, G., Frankenne, F., Closset, F.G., Pirens, G. and El Kyayat, N. (1985) A human placental GH: increasing levels during second half of pregnancy with pituitary GH suppression as re-

vealed by monoclonal antibody radioimmunoassays. *International Journal of Fertility*, **30**, 27–33.

Hensleigh, P.A., Cheatum, S.G. and Spellacy, W.M. (1977) Oxytocinase and human placental lactogen for prediction of intrauterine growth retardation. *American Journal of Obstetrics and Gynecology*, **129**, 675–678.

Hercz, P., Siklos, P., Ungar, L., Farquharson, R.G., Mohari, K. and Kocsar, L. (1987) Change of serum hPL level in maternal vein, umbilical cord vein and artery in mature and premature labour. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **24**, 189–193.

Herington, A.C., Graham, J.Y. and Healy, D.L. (1980) The presence of lactogen receptors in human chorion laeve. *Journal of Clinical Endocrinology and Metabolism*, **51**, 1466–1468.

Hershman, J.M., Kojima, A. and Friesen, H.G. (1973) Effect of thyrotropin-releasing hormone on human pituitary thyrotropin, prolactin, placental lactogen and chorionic thyrotropin. *Journal of Clinical Endocrinology and Metabolism*, **36**, 497–501.

Hertogh, R.D., Thomas, K., Hoet, J.J. and Vanderheyder, I. (1976) Plasma concentrations of unconjugated estrone, estradiol-17 beta and estriol, and HCG throughout pregnancy in diabetics and gestational diabetes. *Diabetologica*, **12**, 455–461.

Hertz, J.B. and Schultz-Larsen, P. (1983) Human placental lactogen, pregnancy specific beta-1-glycoprotein and alpha-fetoprotein in serum in threatened abortion. *International Journal of Gynecology and Obstetrics*, **21**, 111–117.

Heyderman, E., Chapman, D.V., Richardson, T.C., Calvert, I. and Rosen, S.W. (1985) Human chorionic gonadotropin and human placental lactogen in extragonadal tumors. *Cancer*, **56**, 2674–2682.

Higashi, K. (1962) Studies on the prolactin-like substance in human placenta. IV. *Endocrinologica Japonica*, **9**, 1–11.

Hill, D.J., Crace, C.J. and Milner, R.D.G. (1985) Incorporation of 3-H thymidine by isolated fetal myoblasts and fibroblasts in response to human placental lactogen (hPL): possible mediation of hPL action by release of immunoreactive SM-C. *Journal of Cell Physiology*, **125**, 337–344.

Hill, D.J., Freemark, M., Strain, A.J., Handwerger, S. and Milner, R.D.G. (1988) Placental lactogen and growth hormone receptors in human fetal tissues: relationship to fetal plasma hPL concentrations and fetal growth. *Journal of Clinical Endocrinology and Metabolism*, **66**, 1283–1290.

Hirt, H., Kimelman, J., Birnbaum, M.J., Chen, E.Y., Seeburg, P.H., Eberhardt, N.L. and Barta, A. (1987) The human growth hormone locus: structure, evolution and allelic variations. *DNA*, **6**, 59–70.

Hobson, B. and Wide, L. (1972) A comparison between chorionic gonadotropins extracted from human, rhesus monkey and marmoset placentae. *Journal of Endocrinology*, **55**, 363–368.

Hodgen, G.D., Chen, H.C., Dufau, M.L., Klein, T.A. and Mishell, D.R. (1978) Transitory hCG-like activity in the urine of some IUD users. *Journal of Clinical Endocrinology*, **46**, 698–702.

Hogarth, P.J. (1982) *Immunological Aspects of Mammalian Reproduction*, Blackie, Glasgow.

Hoshina, M., Boothby, M. and Boime, I. (1982) Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. *Journal of Cell Biology*, **93**, 190.

Houghton, D.J., Newnham, J.P., Lo, K., Rice, A. and Chard, T. (1982) Circadian variation of circulating levels of four placental proteins. *British Journal of Obstetrics and Gynaecology*, **89**, 831–835.

Houghton, D.J., Shackleton, P., Obiekwe, B.C. and Chard, T. (1984) Relationship of maternal and fetal levels of human placental lactogen to weight and sex of the fetus. *Placenta*, **5**, 455–458.

Huang, S.C., Chen, R.J., Hsieh, C.Y., Wei, P.Y. and Ouyang, P.C. (1984) The secretion of human chorionic gonadotrophin-like substance in women employing contraceptive measures. *Journal of Clinical Endocrinology and Metabolism*, **58**, 646–653.

Hubert, C., Moudon, F. and Cedard, L. (1981) Distribution, quantification and biological activity of messenger RNA coding for human chorionic somatomammotropin during normal pregnancy. *Molecular and Cellular Endocrinology*, **24**, 339–355.

Hubert, C., Descombey, D., Mondon, F. and Daffos, F. (1983) Plasma human chorionic somatomammotropin deficiency in a normal pregnancy is the consequence of low concentration of messenger RNA, coding for human chorionic somatomammotropin. American *Journal of Obstetrics and Gynecology*, **147**, 676–678.

Hunt, R.E., Moffatt, K. and Goldes, D.W. (1981) Purification and crystallization of the polypeptide hormone human chorionic somatomammo-

tropin. *Journal of Biological Chemistry*, **256**, 7042–7045.

Huot, R.I., Foidart, J.M., Nardone, R.M. and Stromberg, K. (1981) Differential modulation of human chorionic gonadotropin secretion by epidermal growth factor in normal and malignant placental cultures. *Journal of Clinical Endocrinology*, **53**, 1059–1063.

Hussa, R.O. (1987) *The Clinical Marker HCG*, Praeger Publishers, Westport, USA.

Hussa, R.O., Rinke, M. and Schweitzer, P.G. (1985) Discordant human chorionic gonadotropin results: causes and solutions. *Obstetrics and Gynecology*, **65**, 211–219.

Huyler, S.E., Butler, W.R., Grandis, A., Mann, C., Figueroa, J.P., Nathanielsz, P.W., Mitchell, M. and Handwerger, S. (1985) Stimulation of ovine placental lactogen secretion by arachidonic acid. *Endocrinology*, **106**, 43–47.

Ikonicoff de, L.K. and Cedard, L. (1973) Localization of human chorionic gonadotropin and somatomammotropic hormones by the peroxidase immunohistoenzymologic method in villi and amniotic epithelium of human placentas (from six weeks to term). *American Journal of Obstetrics and Gynecology*, **116**, 1124–1132.

Iles, R.K. and Chard, T. (1989a) Enhancement of ectopic beta-human chorionic gonadotrophin expression by interferon. *Journal of Endocrinology*, **123**, 501–507.

Iles, R.K. and Chard, T. (1989b) Immunochemical analysis of the human chorionic gonadotrophin-like material secreted by 'normal' and neoplastic urothelial cells. *Journal of Molecular Endocrinology*, **2**, 107–112.

Iles, R.K. and Chard, T. (1991) Human chorionic gonadotrophin expression by bladder cancers: biology and clinical potential. *Journal of Urology*, **145**, 453–458.

Iles, R.K., Czepulkowski, B.H., Young, B.D. and Chard, T. (1989) Amplification or rearrangement of the beta-human chorionic gonadotrophin (beta-hCG)–human LH-gene cluster is not responsible for the ectopic production of beta-hCG by bladder tumour cells. *Journal of Molecular Endocrinology*, **2**, 113–117.

Iles, R.K., Lee, C.L., Howes, I., Davies, S., Edwards, R. and Chard, T. (1992) Immunoreactive beta-core-like material in normal postmenopausal urine: human chorionic gonadotrophin or LH origin? Evidence for the existence of LH core. *Journal of Endocrinology*, **133**, 459–466.

Iles, R.K., Purkis, P.E., Whitehead, P.C., Oliver, R.T.D., Leigh, I. and Chard, T. (1990) Expression of beta human chorionic gonadotrophin by non-trophoblastic non-endocrine 'normal' and malignant epithelial cells. *British Journal of Cancer*, **61**, 663–666.

Imakawa, K., Anthony, R.V., Kazemi, M., Marotti, K.R., Polites, H.G. and Roberts, R.M. (1987) Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. *Nature*, **330**, 377–379.

Ito, Y. and Higashi, K. (1961) Studies on the prolactin-like substance in human placenta. II. *Endocrinologica Japonica*, **8**, 279–287.

Ito, Y. and Higashi, K. (1974) The discovery of human placental lactogen. *American Journal of Obstetrics and Gynecology*, **120**, 427–430.

Jackson, L.L., Colosi, P., Talamantes, F. and Linzer, D.I. (1986) Molecular cloning of mouse placental lactogen cDNA. *Proceedings of the National Academy of Sciences of the USA*, **83**, 8496–8500.

Jameson, J.L. and Lindell, C.M. (1988) Isolation and characterization of the human chorionic gonadotropin β-subunit gene cluster: regulation of a transcriptionally active CGB gene by cyclic AMP. *Molecular and Cellular Biology*, **8**, 5100–5107.

Jameson, J.L., Lindell, C.M. and Habener, J.F. (1987) Gonadotropin and thyrotropin and subunit gene expression in normal and neoplastic tissues characterized by using specific messenger ribonucleic acid hybridization probes. *Journal of Clinical Endocrinology and Metabolism*, **64**, 319–326.

Jepson, J.J. and Friesen, H.G. (1968) The mechanism of action of human placental lactogen on erythropoiesis. *British Journal of Haematology*, **15**, 465–471.

Jones, H.W., Acosta, A.A., Andrews, M.C., Gardia, J.E., Jones, G.S. and Sandow, B.A. (1983) What is pregnancy? A question for *in vitro* fertilisation. *Fertility and Sterility*, **40**, 728–733.

Josimovich, J.B. (1966) Potentiation of somatotrophic and diabetogenic effects of growth hormone by placental lactogen (HPL). *Endocrinology*, **78**, 707–714.

Josimovich, J.B. and Archer, D.F. (1977) The role of lactogenic hormones in the pregnant woman and the fetus. *American Journal of Obstetrics and Gynecology*, **129**, 777–780.

Josimovich, J.B. and Brande, B.L. (1964) Chemical properties and biologic effects of human pla-

cental lactogen (HPL). *Annals of the New York Academy of Science*, **27**, 161–166.

Josimovich, J.B. and MacLaren, J.A. (1962) Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. *Endocrinology*, **71**, 209–220.

Josimovich, J.B., Stock, R.J. and Tobon, H. (1974) Effects of primate placental lactogen upon lactation, in *Lactogenic Hormones, Fetal Nutrition and Lactation*, (ed. J.B. Josimovich), John Wiley & Sons, New York, pp.335–350.

Jouppila, P., Tapanainen, J. and Huhtaniemi, I. (1979) Plasma hCG levels in patients with bleeding in the first and second trimesters of pregnancy. *British Journal of Obstetrics and Gynaecology*, **86**, 343–349.

Jovanovic, L., Dawood, M.Y., Landesman, R. and Saxena, B.B. (1978) Hormonal profile as a prognostic index of early threatened abortion. *American Journal of Obstetrics and Gynecology*, **130**, 274–278.

Julier, C., Weil, D., Couillin, P., Cote, J.C., Nguyen, V.C., Foubert, C., Boue, A., Thirion, J.P., Kaplan, J.C. and Junien, C. (1984) The β-chorionic gonadotropin–β-luteinizing gene cluster maps to human chromosomes. *Human Genetics*, **67**, 174–177.

Kaetzel, D.M. and Nilson, J.H. (1988) Methotrexate-induced amplification of the bovine lutropin genes in Chinese hamster ovary cells: relative concentration of the α and β-subunits determines the extent of heterodimer assembly. *Journal of Biological Chemistry*, **263**, 6344–6351.

Kahn, R.C., Rosen, S.W., Weintraub, S.D., Fajan, S.S. and Gordon, P. (1977) Ectopic production of chorionic gonadotrophin and its subunits by islet cell tumors. *New England Journal of Medicine*, **297**, 565–569.

Kalkhoff, R.K., Richardson, B.L. and Beck, P. (1969) Relative effects of pregnancy, human placental lactogen and prednisolone on carbohydrate tolerance in normal and subclinical diabetic subjects. *Diabetes*, **18**, 153–175.

Kaplan, S. and Grumbach, M.M. (1965) Serum chorionic 'growth hormone–prolactin' and serum pituitary growth hormone in mother and fetus at term. *Journal of Clinical Endocrinology*, **25**, 1370–1374.

Kaplan, S.L., Gurpide, E., Sciarra, J.J. and Grumbach, M.M. (1968) Metabolic clearance rate and production rate of chorionic growth hormone–prolactin in late pregnancy. *Journal of Clinical Endocrinology*, **28**, 1450–1460.

Kardana, A., Taylor, M.E., Southall, J.P., Boxer, G.M., Rowan, A.J. and Bagshawe, K.D. (1988) Urinary gonadotropin peptide – isolation and purification, and its immunohistochemical distribution in normal and neoplastic tissues. *British Journal of Cancer*, **58**, 281–286.

Kato, Y. and Braunstein, G.D. (1988) Beta-core fragment is a major form of immunoreactive urinary chorionic gonadotropin in human pregnancy. *Journal of Clinical Endocrinology and Metabolism*, **66**, 1197–1201.

Katzman, P.A. and Doisy, E.A. (1934) The quantitative determination of small amounts of gonadotropin material. *Journal of Biological Chemistry*, **106**, 125–139.

Katzman, P.A., Gotfrid, M., Cain, C.K. and Doisy, E.A. (1943) The preparation of chorionic gonadotropins by chromatographic adsorption. *Journal of Biological Chemistry*, **148**, 501–507.

Kazer, R.R., Cheng, E.R., Unterman, T.C. and Glick, R.P. (1991) Maternal plasma concentrations of insulin-like growth factor-I (IGF-I) and human placental lactogen (hPL) in twin pregnancies. *Acta Geneticae Medicae et Gemellologiae*, **40**, 383–387.

Keene, J.L., Matzuk, M.M. and Boime, I. (1989) Expression of recombinant human choriogonadotropin in Chinese hamster ovary glycosylation mutants. *Molecular Endocrinology*, **3**, 2011–2017.

Keller, P.J., Baertschi, U., Bader, P., Gerber, C., Schmid, J., Solterman, R. and Kopper, E. (1971) Biochemical detection of fetoplacental distress in risk pregnancies. *Lancet*, **2**, 729–731.

Kelly, A.M., England, P., Lorrimer, J.D., Fergusson, J.C. and Govan, A.D.T. (1975) An evaluation of human placental lactogen levels in hypertension of pregnancy. *British Journal of Obstetrics and Gynaecology*, **82**, 272–277.

Kelly, P.A., Shiu, R.P.C. and Friesen, H.G. (1976) Characterization of rat chorionic mammotropin. *Endocrinology*, **96**, 1187–1197.

Kennedy, J.F. and Chaplin, M.F. (1976) The structures of the carbohydrate moieties of the α-subunit of human chorionic gonadotrophin. *Biochemical Journal*, **155**, 303–315.

Kessler, M.J., Reddy, M.S., Shah, R.H. and Bahl, O.P. (1979a) Structures of *N*-glycosidic carbohydrate subunits of human chorionic gonadotropin. *Journal of Biological Chemistry*, **254**, 7901–7908.

Kessler, M.J., Mise, J., Ghai, R.D. and Bahl, O.P. (1979b) Structure and location of the *O*-glycosidic carbohydrate units of human chorionic gonadotropin. *Journal of Biological Chemistry*, **254**, 7909–7914.

Keutman, H.T., Dawson, B., Bishop, W.H. and Ryan, R.J. (1978) Structure of human luteinizing hormone alpha subunit. *Endocrine Research Communications*, **5**, 57–70.

Keutman, H.T., Charlesworth, M.C., Mason, K.A., Ostrea, T., Johnson, L. and Ryan, R.J. (1987) A receptor-binding region in human choriogonadotropin/lutropin β-subunit. *Proceedings of the National Academy of Sciences of the USA*, **84**, 2038–2042.

Khazaeli, M.B., Hedayat, M.M., Hatch, K.D., To, A.C.W., Soong, S.j., Shingleton, H.M., Boots, L.R. and LoBuglio, A.F. (1989) Radioimmunoassay of free β-subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. *American Journal of Obstetrics and Gynecology*, **155**, 320–324.

Khodr, G. and Siler-Khodr, T.M. (1978) The effect of luteinizing hormone-releasing factor on human chorionic gonadotrophin secretion. *Fertility and Sterility*, **30**, 301–304.

Kim, Y.J. and Felig, P. (1971) Plasma chorionic somatomammotropin levels during starvation in mid-pregnancy. *Journal of Clinical Endocrinology and Metabolism*, **32**, 864–867.

Kim, S.J., Namkoong, S.E., Lee, O.W., Jung, U.K., Kang, B.C. and Oarj, J.S. (1987) Response of human chorionic gonadotrophin to luteinizing hormone-releasing hormone stimulation in the culture media of normal human placenta choriocarcinoma cell lines, and in the serum of patients with gestational trophoblastic disease. *Placenta*, **8**, 257–264.

Kishi, K., Hirashiba, M. and Hasegawa, Y. (1992) Multiple forms of rat placental lactogen-II (rPL-II): purification and partial characterization of rPL-II. *Placenta*, **13**, 63–79.

Klassen, M.E., Nachtigal, M.W. and Cattini, P.A. (1989) Human chorionic somatomammotropin gene expression in primary placental cell cultures. *Placenta*, **10**, 321–329.

Klebe, J.G., Marushak, A. and Bock, J. (1990) Human placental lactogen lactogenic hormone as a parameter for placental function in renal transplanted women. *Acta Obstetrica et Gynecologica Scandinavica*, **69**, 41–43.

Kliman, H.J., Nestler, J.E., Sermasi, E., Sanger, J.M. and Strauss, J.F. (1986) Purification, characterization and *in vitro* differentiation of cytotrophoblasts from human term placentae. *Endocrinology*, **118**, 1567–1582.

Klopper, A. and Hughes, G. (1978) Placental secretion of oestrogens and protein hormones. *Archives of Gynecology*, **225**, 171–177.

Knoll, B.J. (1992) Gene expression in the human placental trophoblast: a model for developmental gene regulation. *Placenta*, **13**, 311–327.

Kobata, A. (1988) Altered glycosylation of human chorionic gonadotropin in malignant trophoblastic diseases, in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 133–142.

Kofler, E. and Spona, J. (1971) Hormone studies of an ovarian dysgerminoma. *American Journal of Obstetrics and Gynecology*, **111**, 1028–1033.

Kulski, J.K., Ethel, P., Hartmann, P.E. and Smith, M. (1982) Placental lactogen in human serum and colostrum. *Hormone and Metabolic Research*, **14**, 441–442.

Lagrew, D.C., Wilson, E.A. and Fried, A.M. (1984) Accuracy of serum human chorionic gonadotropin concentrations and ultrasonic fetal measurements in determining gestational age. *American Journal of Obstetrics and Gynecology*, **149**, 165–168.

Landesman, R., Continho, E.M. and Saxena, B.B. (1976) Detection of hCG in blood of regularly bleeding women using copper intrauterine contraceptive devices. *Fertility and Sterility*, **27**, 1062–1064.

Larinkari, J., Laatikainen, L., Ranta, T., Moronen, P. and Pesonen, K. (1982) Metabolic control and serum hormone levels in relation to retinopathy in diabetic pregnancy. *Diabetologica*, **22**, 327–332.

Laurin, J., Persson, P.H. and Fernlund, P. (1987) The efficacy of biochemical assays in assessing an IUGR risk-group, preselected with ultrasound fetometry. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **24**, 177–187.

Lee, C.L., Shepherd, J.H., Hudson, C.N. and Chard, T. (1991) The purification and development of a radioimmunoassay for beta-core fragment of human chorionic gonadotrophin in urine: application as a marker of gynaecological cancer in premenopausal and postmenopausal women. *Journal of Endocrinology*, **130**, 481–489.

Lee, J.N., Grudzinskas, J.G. and Chard, T. (1980) Circulating human placental lactogen (HPL) levels in relation to smoking during pregnancy. *Journal of Obstetrics and Gynaecology*, **1**, 87–89.

Lee, J.N., Seppala, M. and Chard, T. (1981) The characterization of placental luteinizing hormone releasing factor-like material. *Acta Endocrinologica*, **96**, 394–397.

Lee, J.N., Wu, P. and Chard, T. (1982) Identification of somatostatin in the human placenta. *Acta Endocrinologica*, **99**, 601–604.

Lee, J.N., Lian, D.A., Lee, J.H. and Chard, T. (1983) Placental proteins (human chorionic gonadotrophin, human placental lactogen, pregnancy-specific beta-1 glycoprotein, and placental protein 5) in seminal plasma of normal men and patients with infertility. *Fertility and Sterility*, **39**, 704–706.

Leigh, S.E.A. and Stewart, F. (1990) Partial cDNA sequence for the donkey chorionic gonadotropin-β-subunit suggests evolution from an ancestral LH-βgene. *Journal of Endocrinology*, **4**, 143–150.

Letchworth, A.T. (1976) Human placental lactogen assay as a guide to fetal wellbeing, in *Plasma Hormone Assays in Evaluation of Fetal Wellbeing*, (ed. A. Klopper), Churchill Livingstone, Edinburgh, pp. 143–173.

Letchworth, A.T. and Chard, T. (1972a) Human placental lactogen in pre-eclampsia. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **79**, 680.

Letchworth, A.T. and Chard, T. (1972b) Placental lactogen levels as a screening test for fetal distress and neonatal asphyxia. *Lancet*, **1**, 704.

Letchworth, A.T., Boardman, R., Bristow, C., Landon, J. and Chard, T. (1971) A rapid radioimmunoassay for human chorionic somatomammotrophin. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **78**, 535.

Letchworth, A.T., Howard, L. and Chard, T. (1974) Placental lactogen levels during bed rest. *Obstetrics and Gynecology*, **43**, 702–703.

Li, C.H., Grumbach, M., Kaplan, S.L., Josimovich, J.B., Friesen, H. and Catt, K.J. (1968) Human chorionic somatotropin (HCS), proposed terminology for designation of a placental hormone. *Experientia*, **24**, 1288.

Light, P.A., Foster, J.P., Feltou, T., Eckert, H. and Tovey, K.C. (1983) Molecular heterogeneity of chorionic gonadotropin in some testicular cancer patients. *Lancet*, **ii**, 1284.

Lilford, R.J., Obiekwe, B.C. and Chard, T. (1983) Maternal blood levels of human placental lactogen in the prediction of fetal growth retardation: choosing a cut-off point between normal and abnormal. *British Journal of Obstetrics and Gynaecology*, **90**, 511–515.

Lin, T.M. and Halbert, S.P. (1978) Immunological relationships of human and subhuman primate pregnancy-associated plasma proteins. *International Archives of Allergy and Applied Immunology*, **56**, 207–223.

Lindberg, B.S., Martinsson, K., Lindberg, P., Nilsson, B.A. and Johansson, E.D.B. (1973) Variations in maternal plasma levels of HPL in normal pregnancy and labour. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 619–626.

Lindberg, B.S. and Nilsson, B.A. (1973) hPL levels in abnormal pregnancies. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 1046–1053.

Lolis, D., Kostantinidis, K., Papevangelou, G. and Kaskarelis, D. (1977) Comparative study of amniotic fluid and maternal blood serum human placental lactogen in normal and prolonged pregnancies. *American Journal of Obstetrics and Gynecology*, **128**, 724–726.

Loosfelt, H., Misrahi, M., Atger, M., Salesse, R., Thi, M.T.V.H.L., Jolivet, A., Guiochon-Mantel, A., Sar, S., Jallal, B., Garnier, J. and Milgrom, E. (1989) Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain. *Science*, **245**, 525–528.

Lopata, A. and Hay, D.L. (1988) Chorionic gonadotropin secretion by human embryos *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **67**, 1322–1324.

Lopez, M.F., Carrion, F.A. and Talamantes, F. (1991) Pituitary-placental interaction: hypophysectomy modulates the secretion of mouse placental lactogen-I. *Endocrinology*, **129**, 2325–2328.

Louvet, J.P., Ross, G.T., Birken, S. and Canfield, R.E. (1974) Absence of neutralizing effect of antisera to the unique structural region of human chorionic gonadotropin. *Journal of Clinical Endocrinology and Metabolism*, **39**, 1155–1158.

Lowman, H.B., Cunningham, B.C. and Wells, J.A. (1991) Mutational analysis and protein engineering of receptor-binding determinants in human placental lactogen. *Journal of Biological Chemistry*, **266**, 10982–10988.

Lustbader, J.W., Birken, S., Pileggi, N.F., Gawinowicz Kolks, M.A., Pollak, S., Cuff, M.E., Yang, W., Hendrickson, W.A. and Canfield, R.E. (1989) Crystallization and characterization of human chorionic gonadotropin in

chemically deglycosylated and enzymatically desialylated states. *Biochemistry*, **28**, 9239–9243.

Macaron, C., Famuyiwa, O. and Sing, P. (1978) *In vitro* effect of dopamine and pimozide on human chorionic somatomammotropin (hCG) secretion. *Journal of Clinical Endocrinology and Metabolism*, **47**, 168–170.

MacDonald, D.J., Nicol, K.M., Belfield, A., Shah, M.M. and Mack, D.S. (1980) Enzyme-linked immunoassay for placental lactogen in human serum. *Clinical Chemistry*, **26**, 745–747.

MacDonald, D.J., Scott, J.M., Gemmell, R.S. and Mack, D.S. (1983) A prospective study of three biochemical fetoplacental tests: serum human placental lactogen, pregnancy-specific beta-1 glycoprotein and urinary estrogens, and their relationship to placental insufficiency. *American Journal of Obstetrics and Gynecology*, **147**, 430–436.

McFarland, K.C., Sprengel, R., Phillips, H.S., Kohler, M., Rosemblit, N., Nikolics, K., Segaloff, D.L. and Seeburg, A. (1989) Lutropin–choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. *Science*, **245**, 494–499.

McFadzean, A.J.S. (1946) Feminisation associated with carcinoma of the adrenal cortex. *Lancet*, **2**, 940–943.

McManus, L.M., Naughton, M.A. and Martinez-Hernandez, A.M. (1976) Human chorionic gonadotropin in human neoplastic cells. *Cancer Research*, **36**, 3476–3481.

McWilliams, D. and Boime, I. (1980) Cytological localization of placental lactogen messenger ribonucleic acid in syncytiotrophoblast layers of human placenta. *Endocrinology*, **10**, 761–765.

Mann, K., Schneider, N. and Hoerman, R. (1986) Thyrotropic activity of acidic isoelectric variants of human chorionic gonadotropin from trophoblastic tumours. *Endocrinology*, **118**, 1558–1566.

Marcillac, I., Troalen, F., Bidart, J.M., Ghillani, P., Ribrag, V., Escudier, B., Malassagne, B., Droz, J.P., L'homme, C., Rougier, P., Duvillard, P., Pade, M., Lugagne, P.M., Richard, F., Bohuon, C., Wands, J. and Bellet, D. (1992) Free human chorionic gonadotropin β subunit in gonadal and nongonadal neoplasms. *Cancer Research*, **52**, 3901–3907.

Markkanen, S.O., Tollikko, K., Vanha-Perttula, T. and Rajaniemi, H. (1979) Disappearance of human 125-I-iodochorionic gonadotropin from the circulation of the rat: tissue uptake and degradation. *Endocrinology*, **104**, 1540–1547.

Markoff, E. and Talamantes, F. (1981) Serum placental lactogen in mice in relation to day of gestation and number of conceptuses. *Biology of Reproduction*, **24**, 846–849.

Marsh, J.M. and LeMaire, W.J. (1974) Cyclic AMP accumulation and steroidogenesis in the human corpus luteum: effect of gonadotropins and prostaglandins. *Journal of Clinical Endocrinology and Metabolism*, **38**, 99–106.

Martin, J.E., Jenkins, B.J., Zuk, R.J., Oliver, R.T.D. and Baithun, S.I. (1989) Human chorionic gonadotrophin expression and histological findings as predictors of response to radiotherapy in carcinoma of the bladder. *Virchows Archives A (Pathological Anatomy and Histopathology)*, **414**, 273–277.

Martin, J.M. and Friesen, H. (1969) Effect of human placental lactogen on the isolated islets of Langerhans *in vitro*. *Endocrinology*, **84**, 619–621.

Maruo, T., Matsuo, H., Ohtani, T., Hoshina, M. and Mochizuki, M. (1986) Differential modulation of chorionic gonadotropin (CG) subunit messenger ribonucleic acid levels and CG secretion by progesterone in normal placenta and choriocarcinoma cultured *in vitro*. *Endocrinology*, **119**, 855–864.

Maruo, T., Matsuo, H., Murata, K. and Mochizuki, M. (1992) Gestational age-dependent dual action of epidermal growth factor on human placenta early in gestation. *Journal of Clinical Endocrinology and Metabolism*, **75**, 1362–1367.

Masson, G, M., Klopper, A. and Wilson, G.R. (1977) Plasma estrogens and pregnancy-associated plasma proteins. A study of their variability in late pregnancy. *Obstetrics and Gynecology*, **50**, 435–438.

Masson, G.M., Anthony, F., Wilson, M.S. and Lindsay, K. (1983) Comparison of serum and urine hCG levels with SP1 and PAPP-A levels in patients with first trimester vaginal bleeding. *Obstetrics and Gynecology*, **61**, 223–226.

Matsuura, S., Ohashi, M., Chen, H.C., Shownkeen, R.C., Stockell Hartree, A., Reichert, L.E., Stevens, V.C. and Powell, J.E. (1980) Physicochemical and immunological characterization of an hCG-like substance from human pituitary glands. *Nature*, **286**, 740–741.

Matzuk, M.M., Husueh, A.J.W., Lappolt, P., Tsafriri, A., Keen, J.L. and Boime, I. (1990) The biological role of the carboxyl-terminal extension of human chorionic gonadotropin β-subunit. *Endocrinology*, **126**, 376–383.

Medvei, V.C. (1982) *History of Endocrinology*, MTP Press, Lancaster.

Merkatz, J.R., Nitowsky, H.M., Macri, J.N. and Johnson, W.E. (1984) An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *American Journal of Obstetrics and Gynecology*, **14**, 886–894.

Metzger, B.E., Rodeck, C., Freinkel, N., Price, J. and Young, M. (1985) Transplacental arteriovenous gradients for glucose, insulin, glucagon and placental lactogen during normoglycaemia in human pregnancy at term. *Placenta*, **6**, 347–354.

Miller, J.R., Williamson, E., Glue, J., Gordon, Y.B., Grudzinskas, J.G. and Sykes, A. (1980) Fetal loss after implantation. *Lancet*, **2**, 554–556.

Milman, N., Agger, A.O. and Nielsen, O.J. (1991) Iron supplementation during pregnancy. Effect on iron status markers, serum erythropoietin and human placental lactogen. A placebo controlled study in 107 Danish women. *Danish Medical Bulletin*, **38**, 471–476.

Milstead, A., Cox, R.P. and Nilson, H.J. (1987) Cyclic AMP regulates transcription of the genes encoding chorionic gonadotrophin with different kinetics. *DNA*, **6**, 213–219.

Mise, T. and Bahl, O.P. (1980) Assignment of disulphide bonds in the α-subunit of human chorionic gonadotropin. *Journal of Biological Chemistry*, **255**, 8516–8522.

Mise, T. and Bahl, O.P. (1981) Assignment of disulphide bonds in the β-subunit of human chorionic gonadotropin. *Journal of Biological Chemistry*, **256**, 6587–6592.

Mizuochi, T. and Kobata, A. (1980) Different asparagine-linked sugar chains on the two polypeptide chains of human chorionic gonadotropin. *Biochemical and Biophysical Research Communications*, **97**, 772–778.

Mizuochi, T., Nishimura, R., Derappe, C., Taniguchi, T., Hamamoto, T., Mochizuki, M. and Kobata, A. (1983) Structures of asparagine-linked sugar chains of human chorionic gonadotropin produced in choriocarcinoma. Appearance of triantennary sugar chains and unique biantennary sugar chains. *Journal of Biological Chemistry*, **258**, 14126–14129.

Monteiro, J.C.M.P., Barker, G., Ferguson, K.M., Wiltshaw, E. and Munro Neville, A. (1983) Ectopic production of human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) by ovarian carcinoma. *European Journal of Cancer and Clinical Oncology*, **19**, 173–178.

Monteiro, J.C.M.P., Biswas, S., Al-Awqati, M.A., Greening, W.P., McKinna, J.A. and Neville, A.M. (1982) Serum levels of human placental lactogen, and pregnancy-specific beta-1 glycoprotein in breast cancer. *British Journal of Cancer*, **46**, 279–282.

Moore, W.T. and Ward, D.N. (1980) Pregnant mare serum gonadotropin: rapid chromatographic procedures for the purification of intact hormone and isolation of subunits. *Journal of Biological Chemistry*, **255**, 6923–6929.

Morgan, D.A., Caillaud, J.M., Bellet, D. and Eschwege, F. (1982) Gonadotropin-producing seminoma: a distinct category of germ cell neoplasm. *Clinical Radiology*, **33**, 149–153.

Morgan, F.J., Birken, S. and Canfield, R.E. (1975) The amino acid sequence of human chorionic gonadotropin. The alpha subunit and beta subunit. *Journal of Biological Chemistry*, **250**, 5247–5258.

Mori, K.F. (1970) Antigenic structure of human chorionic gonadotropins: importance of protein moiety to the antigenic structure of human chorionic gonadotropin. *Endocrinology*, **86**, 97–106.

Morris, H.H.B, Vinik, A.I. and Mulvihal, M. (1974) Effects of acute alterations in free fatty acid concentration in human chorionic somatomammotropin secretion. *American Journal of Obstetrics and Gynecology*, **119**, 224–229.

Morrish, D.W., Bhardwaj, D. and Paras, M.T. (1991) Transforming growth factor beta 1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. *Endocrinology*, **129**, 22–26.

Morrison, I., Green, P. and Oomen, B. (1980) The role of human placental lactogen assays in antepartum fetal assessment. *American Journal of Obstetrics and Gynecology*, **136**, 1055–1060.

Morse, J.H., Stearns, G., Arden, J., Agosts, G.M. and Canfield, R.E. (1976) The effects of crude and purified human gonadotropin *in vitro* stimulated human lymphocyte cultures. *Cellular Immunology*, **25**, 178–188.

Moser, R.J., Hollingsworth, D.R., Carlson, J.W. and Lamotte, L. (1974) Human chorionic somatomammotropin in normal adolescent primiparous pregnancy. *American Journal of Obstetrics and Gynecology*, **120**, 1080–1806.

Moyle, W.R., Lustbader, J.W. and Canfield, R.E. (1987) Discussion Section of: Ryan RJ, Keutmann HT, Charlesworth MC, McCormick DJ, Milius RP, Calvo FO and Vutyavanich T

(1987) Structure–function relationships of gonadotropins. *Recent Progress in Hormone Research*, **43**, 383–429.

MRC Working Party on Testicular Tumours (1985) Prognostic factors in advanced non-seminomatous germ-cell testicular tumours: results of a multicentre study. *Lancet*, **1**, 8–11.

Muchmore, A.V. and Blaese, R.M. (1977) Immunoregulatory properties of fractions from human pregnancy urine: evidence that human chorionic gonadotropin is not responsible. *Journal of Immunology*, **118**, 881–886.

Murakawa, S. and Raben, M.S. (1968) Effect of growth hormone and placental lactogen on DNA synthesis in rat costal cartilage and adipose tissue. *Endocrinology*, **83**, 645–650.

Nagasawa, H., Yanai, R. and Yamanouchi, K. (1976) Inhibition of pituitary prolactin secretion by human placental lactogen in rats. *Journal of Endocrinology*, **71**, 115–120.

Nam, J.H., Chang, K.C., Chambers, J.T., Schwartz, P.E. and Cole, L.A. (1989) Urinary gonadotropin fragment, a new tumor marker. III. Use in cervical and vulvar cancers. *Gynecological Oncology*, **38**, 66–70.

Nash, H.A., Chang, C.C. and Tsong, Y.Y. (1985) Formulation of a potent anti pregnancy vaccine based on the β-subunit of human chorionic gonadotropin (β-hCG). II. Use of compounds of the muramyl dipeptide (MDP) family as adjuvants. *Journal of Reproductive Immunology*, **7**, 151–162.

Naylor, S.L., Chin, W.W., Goodman, H.M., Lalley, P.A., Grzeschik, K.H. and Sakaguchi, A.Y. (1983) Chromosome assignment of genes encoding the alpha and beta subunits of glycoprotein hormones in man and mouse. *Somatic Cell Genetics*, **9**, 757–770.

Newnham, J.P., Lam, R.W., Hobel, C.J., Padbury, J.F., Polk, D.H. and Fisher, D.A. (1986a) Differential response of ovine placental lactogen levels in maternal and fetal circulations following single umbilical artery ligation in fetal sheep. *Placenta*, **7**, 51–64.

Newnham, J.P., Lam, R.W., Hobel, C.J., Polk, D.H. and Fisher, D.A. (1986b) Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnancy sheep. *American Journal of Obstetrics and Gynecology*, **154**, 663–666.

Niall, H.D., Hogan, M.L., Sauer, R., Rosenblum, I.Y. and Greenwood, F.C. (1971) Sequences of pituitary and placental lactogenic and GH evolution from a primordial peptide by gene reduplication. *Proceedings of the National Academy of Sciences of the USA*, **68**, 866–869.

Niall, H.D., Hogan, M.L., Tregear, G.W., Seagre, G.V., Hwang, P. and Friesen, H.G. (1973) The chemistry of growth hormone and the lactogenic hormones. *Recent Progress in Hormone Research*, **29**, 387–398.

Nicolaides, K.H., Faratian, B. and Symonds, E.M. (1982) Effect of low implantation of the placenta on maternal blood pressure and placental function. *British Journal of Obstetrics and Gynaecology*, **89**, 806–810.

Nielsen, P.V., Pedersen, H. and Kampmann, E.M. (1979) Absence of human placental lactogen in an otherwise uneventful pregnancy. *American Journal of Obstetrics and Gynecology*, **135**, 322–326.

Nilsson, B., Rosen, S.W., Weintraub, B.D. and Zopf, D.A. (1986) Differences in the carbohydrate moieties of the common α-subunits of human chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone and thyrotropin: preliminary structural inferences for direct methylation analysis. *Endocrinology*, **119**, 2737–2743.

Nishimura, R., Endo, Y., Tanabe, K., Ashitaka, Y. and Tojo, S. (1981) The biochemical properties of urinary human chorionic gonadotropin from the patients with trophoblastic disease. *Journal of Endocrinological Investigations*, **4**, 349–358.

Nisula, B. and Bartocci, A. (1984) Choriogonadotropin and immunity: a reevaluation. *Annales d'Endocrinologie (Paris)*, **45**, 315–319.

Nisula, B.C., Blithe, D.L., Akar, A., Lefort, G. and Wehmann, R.E. (1989) Metabolic fate of human choriogonadotropin. *Journal of Steroid Biochemistry*, **33**, 733–737.

Niven, P.A.R., Chard, T. and Landon, J. (1972) Placental lactogen levels as a guide to the outcome of threatened abortion. *British Medical Journal*, **3**, 799.

Niven, P.A.R., Ward, R.H.T. and Chard, T. (1974) Human placental lactogen levels in amniotic fluid in Rhesus isoimmunization. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 988.

Norman, R.J., Poulton, T., Gard, T. and Chard, T. (1985a) Monoclonal antibodies to human chorionic gonadotrophin: implications for antigenic mapping, immunoradiometric assays and clinical applications. *Journal of Clinical Endocrinology and Metabolism*, **61**, 1031–1038.

Norman, R.J., Lowings, C. and Chard, T. (1985b) Dipstick method for human chorionic gonadotropin suitable for emergency use on whole blood and other fluids. *Lancet*, **1**, 19-20.

Norman, R.J., Lowings, C., Oliver, T. and Chard, T. (1985c) Human chorionic gonadotrophin and subunits-heterogeneity in serum of male patients with tumours of the genital tract. *Clinical Endocrinology*, **27**, 25–34.

Novy, M.J., Aubert, M.L., Kaplan, S.L. and Grumbach, M.M. (1981) Regulation of placental growth and chorionic somatomammotropin in the rhesus monkey: effects of protein deprivation, fetal anencephaly and placental vessel ligation. *American Journal of Obstetrics and Gynecology*, **140**, 552–562.

Nygren, K.G., Johansson, E.D.B. and Wide, L. (1973) Evaluation of the prognosis of threatened abortion from the peripheral levels of plasma progesterone, estradiol and human chorionic gonadotropin. *American Journal of Obstetrics and Gynecology*, **116**, 916–922.

Obiekwe, B.C. and Chard, T. (1982a) Human chorionic gonadotrophin levels in maternal blood in late pregnancy: relation to birthweight, sex and condition of the infant at birth. British *Journal of Obstetrics and Gynaecology*, **89**, 543–546.

Obiekwe, B.C. and Chard, T. (1982b) What do placental function tests predict? Observations on placental lactogen levels in growth retardation and fetal distress. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **14**, 69–73.

Obiekwe, B.C. and Chard, T. (1983a) Placental proteins in late pregnancy: relation to fetal sex. *Journal of Obstetrics and Gynaecology*, **3**, 163–164.

Obiekwe, B.C. and Chard, T. (1983b) A comparative study of the clinical use of four placental proteins in the third trimester. *Journal of Perinatal Medicine*, **11**, 121–126.

Obiekwe, B.C. and Chard, T. (1984) The value of serial estimations of placental lactogen for fetoplacental function testing in the diagnosis of intra-uterine growth retardation. *Journal of Obstetrics and Gynaecology*, **4**, 157–160.

Obiekwe, B.C., Sturdee, D., Cockrill, B.L. and Chard, T. (1984) Human placental lactogen in pre-eclampsia. *British Journal of Obstetrics and Gynaecology*, **91**, 1077–1080.

Odell, W.D. and Griffin, J. (1987) Pulsatile secretion of human chorionic gonadotropin in normal adults. *New England Journal of Medicine*, **317**, 1688–1691.

Odell, W.D. and Griffin, J. (1989) Pulsatile secretion of chorionic gonadotropin during the normal menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, **69**, 528–532.

Ogren, L., Southard, J.N., Colosi, P., Linzer, D.I.H. and Talamantes, F. (1989) Mouse placental lactogen-I: RIA and gestational profile in maternal serum. *Endocrinology*, **125**, 2253–2257.

Oliver, M.H., Harding, J.E., Breier, B.H., Evans, P.C. and Gluckman, P.D. (1992) The nutritional regulation of circulating placental lactogen in fetal sheep. *Pediatric Research*, **31**, 520–523.

Otani, T., Otani, F., Bo, M., Chaplin, D. and Boime, I. (1988) Promoter sequences in the chorionic gonadotropin β-subunit gene, in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 87–100.

Pala, A., Meirinho, M. and Benajiano, G. (1973) Purification and properties of chorionic gonadotrophin from trophoblastic tissue, urine and plasma of a patient with hydatidiform mole. *Journal of Endocrinology*, 56, 441–450.

Papapetrou, P.D. and Nicopoulou, S.C. (1986) The origin of a human chorionic gonadotropin β-subunit-core fragment excreted in the urine of patients with cancer. *Acta Endocrinologica*, **112**, 415–422.

Parks, J.S., Nielsen, P.V., Sexton, L.A. and Jorgensen, E.H. (1985) An effect of gene dosage on production of human chorionic somatomammotropin. *Journal of Clinical Endocrinology and Metabolism*, **60**, 994–997.

Parsons, T.F., Bloomfield, G.A. and Pierce, J.G. (1983) Purification of an alternate form of the α-subunit of the glycoprotein hormones from bovine pituitaries and identification of its *O*-linked oligosaccharide. *Journal of Biological Chemistry*, **258**, 240–244.

Parsons, T.F. and Pierce, J.G. (1980) Oligosaccharide moieties of glycoprotein hormones: bovine lutropin resists enzymatic deglycosylation because of terminal *O*-sulfated *N*-acetyl hexosamines. *Proceedings of the National Academy of Sciences of the USA*, 77, 7089–7093.

Parsons, T.F. and Pierce, J.G. (1984) Free α-like material from bovine pituitaries. Removal of its *O*-linked oligosaccharide permits combination with lutropin-B. *Journal of Biological Chemistry*, **259**, 2662–2666.

Patillo, R.A., Shalby, M.R., Hussa, R.O., Bahl, O.P. and Mattingly, R.F. (1976) Effect of crude

and purified hCG on lymphocyte blastogenesis. *Obstetrics and Gynecology*, **47**, 557–561.

Pavlou, C., Chard, T. and Letchworth, A.T. (1972) Circulating levels of human chorionic somnatomammotrophin in late pregnancy: disappearance from the circulation after delivery, variation during labour, and circadian rhythm. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **79**, 629.

Pavlou, C., Chard, T., Landon, J. and Letchworth, A.T. (1973) Circulating levels of human placental lactogen in late pregnancy: the effects of glucose loading, smoking and exercise. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, **3**, 45.

Peters, B.P., Krzesicki, R.F., Perini, F. and Ruddon, R.W. (1989) *O*-glycosylation of the alpha-subunit does not limit the assembly of chorionic gonadotropin alpha beta dimer in human malignant and nonmalignant trophoblastic cells. *Endocrinology*, **124**, 1602–1612.

Petit, A., Guillon, G., Tence, M., Jard, S., Gallo-Payet, N., Bellabarba, D., Lehoux, J.G. and Belisle, S. (1989) Antiotensin II stimulates both inositol phosphate production and human placental lactogen release from human trophoblastic cells. *Journal of Clinical Endocrinology and Metabolism*, **69**, 280–286.

Pierce, J.G. and Parsons, T.F. (1981) Glycoprotein hormones: structure and function. *Annual Reviews of Biochemistry*, **50**, 465–495.

Pilistine, S.J., Moses, A.C. and Munro, H.N. (1984) Placental lactogen administration reverses the effect of low-protein diet on maternal and fetal serum somatomedin levels in the pregnant rat. *Proceedings of the National Academy of Sciences of the USA*, **81**, 5853–5857.

Pittaway, D.E., Reish, M.S. and Wentz, A.C. (1985a) Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. *American Journal of Obstetrics and Gynecology*, **152**, 299–302.

Pittaway, D.E., Wentz, A.C., Maxon, W.S., Herbert, C., Daniell, J. and Fleischer, A.C. (1985b) The efficacy of early pregnancy monitoring with serial chorionic gonadotropin determinations and real-time ultrasonography in an infertile population. *Fertility and Sterility*, **44**, 190–194.

Pledger, D.R., Lunan, C.B. and Belfield, A. (1982) Human placental lactogen and pregnancy specific-beta-1 glycoprotein in pregnant diabetic women. *British Journal of Obstetrics and Gynaecology*, **89**, 827–830.

Pledger, D.R., Belfield, A., Calder, A.A. and Wallace, A.M. (1984) The predictive value of three pregnancy-associated proteins in the detection of the light-for-dates baby. *British Journal of Obstetrics and Gynaecology*, **91**, 870–874.

Policastro, P., Ovitt, C.E., Hoshina, M., Fukuoka, H., Noothby, M.R. and Boime, I. (1983) The β-subunit of human chorionic gonadotropin is encoded by multiple genes. *Journal of Biological Chemistry*, **258**, 11492–11499.

Policastro, P.F., Daniels-McQueen, S., Carle, G. and Boime, I. (1986) A map of the hCGβ–LHβ cluster. *Journal of Biological Chemistry*, **261**, 5907–5916.

Porter, D.G. (1980) Review Article: Feto-maternal relationships: The actions and control of certain placental hormones. *Placenta*, **1**, 259–274.

Prieto, J.C., Cifuentes, I. and Serrano-Rios, M. (1976) hCS regulation during pregnancy. *Obstetrics and Gynecology*, **48**, 287–301.

Quigley, M.M., Tyrey, L. and Hammond, C.B. (1980a) α-subunit in sera of choriocarcinoma patients in remission. *Journal of Clinical Endocrinology and Metabolism*, **50**, 98–102.

Quigley, M.M., Tyrey, L. and Hammond, C.B. (1980b) Utility of assay of alpha subunit of human chorionic gonadotropin in management of gestational, trophoblastic malignancies. *American Journal of Obstetrics and Gynecology*, **138**, 545–549.

Ranta, T., Sitteri, J.E. and Koistinen, R. (1981) Human seminal plasma contains a protein that shares physicochemical and immunochemical properties with placental protein 5 from the human placenta. *Journal of Clinical Endocrinology and Metabolism*, **53**, 1087–1089.

Ranta, T., Chen, H.C., Jalkanen, J., Nikula, H., Shimohigashi, Y. and Huhtaniemi, I. (1987) Receptor binding properties and biologic action of deglycosylated human chorionic gonadotropin in human ovary and testis. *Obstetrics and Gynecology*, **70**, 171–174.

Redman, C.W.G. (1986) Immunology of the placenta, in *The Human Placenta. Clinical Obstetrics and Gynecology*, (ed. T. Chard), W.B. Saunders, London, pp. 469–499.

Reeve, J.R., Cheng, K.W. and Pierce, J.G. (1975) Partial reduction of disulphide bonds in the hormone specific subunits of TSH and LH. *Biochemical and Biophysical Research Communications*, **67**, 149–155.

Rice, G.E. and Thorburn, G.D. (1986) Characterization of particle associated choriomammotrophin and progesterone in ovine placentomes. *Journal of Endocrinology*, **111**, 217–223.

Richardson, M.C. and Masson, G.M. (1981) Stimulation by human chorionic gonadotropin of oestradiol production by dispersed cells from human corpus luteum: comparison with progesterone production; utilization of exogenous testosterone. *Journal of Endocrinology*, **91**, 197–203.

Riggi, S.J., Boshart, C.R., Bell, P.H. and Ringler, I. (1966) Some effects of purified placental protein (human) on lipid and carbohydrate metabolism. *Endocrinology*, **79**, 709–715.

Robertson, D.M., Suginami, H., Montes, H.H., Puri, C.P. and Choi, S.K. (1978) Studies on a human chorionic gonadotrophin-like material present in non-pregnant subjects. *Acta Endocrinologica*, **89**, 492–505.

Robertson, M.C., Gillespie, B. and Friesen, H.G. (1982) Characterization of the two forms of rat placental lactogen (rPL): rPL-I and rPL-II. *Endocrinology*, **111**, 1862–1866.

Robertson, M.C., Owens, R.E. and Friesen, H.G. (1984) Ovariectomy leads to a rapid increase in rat placental lactogen secretion. *Endocrinology*, **114**, 1805–1811.

Romero, R., Kadar, N., Copel, J.A., Jeanty, P., DeCherney, A.H. and Hobbins, J.C. (1985a) The effect of different human chorionic gonadotropin assay sensitivity on screening for ectopic pregnancy. *American Journal of Obstetrics and Gynecology*, **153**, 72–74.

Romero, R., Kadar, N., Jeanty, P., Copel, J.A., Chervenak, F.A., DeCherney, A. and Hobbins, J.C. (1985b) Diagnosis of ectopic pregnancy: value of the discriminatory human chorionic gonadotropin zone. *Obstetrics and Gynecology*, **66**, 357–360.

Rosa, C., Amr, S., Birken, S., Wehmann, R. and Nisula, B. (1984) Effect of desialylation of human chorionic gonadotropin on its metabolic clearance rate in humans. *Journal of Clinical Endocrinology and Metabolism*, **59**, 1215–1219.

Rosen, S.W., Weintraub, B.D. and Aaronson, S.A. (1980a) Nonrandom ectopic protein production by malignant cells: direct evidence *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **50**, 834–841.

Rosen, S., Kaminska, J., Calvert, I., Ellmore, N. and Aaronson, S. (1980b) Ectopic production of 'pregnancy-specific' beta-1 glycoprotein *in vitro*: discordance with three other placental proteins. *American Journal of Obstetrics and Gynecology*, **137**, 525–529.

Ruddon, R.W., Anderson, C., Meade-Cobun, K.S., Aldenderfer, P.H. and Neuwald, P.D. (1979a) Content of gonadotropins in cultured human malignant cells and effects of sodium butyrate treatment on gonadotropin secretion by HeLa cells. *Cancer Research*, **39**, 3885–3892.

Ruddon, R.W., Hanson, C.A. and Addison, N.J. (1979b) Synthesis and processing of human chorionic gonadotropin subunits in cultured choriocarcinoma cells. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5143–5147.

Ruddon, R.W., Krzesicki, R.F., Norton, S.E., Saccuzzo-Beebe, J., Peters, B.P. and Perini, F. (1987) Detection of glycosylated incompletely folded form of chorionic gonadotropin beta subunit that is a precursor of hormone assembly in trophoblastic cells. *Journal of Biological Chemistry*, **262**, 12533–12540.

Ruddon, R.W., Krzesicki, R.F., Loesel, L., Saccuzzo-Beebe, J., Peters, B.P. and Perini, F. (1988) Biosynthesis and combination of chorionic gonadotropin subunits in human cells, in *Proceedings of the Satellite Symposium on Placental Protein Hormones (Japan)*, (eds M. Mochilzuki and R. Hussa), Excerpta Medica, Amsterdam, pp. 75–85.

Russell, J., Sherwood, L.M., Kowalski, K. and Schneider, A.R. (1981a) Recombinant hormones from fragments of human growth hormone and human placental lactogen. *Journal of Biological Chemistry*, **256**, 296–300.

Russell, J., Katzhendler, J., Kowalski, K., Schneider, A.B. and Sherwood, L.M. (1981b) The single tryptophan residue of human placental lactogen. *Journal of Biological Chemistry*, **256**, 304–307.

Rutanen, E.M. (1978) The circulating alpha subunit of human chorionic gonadotropin in gynaecologic tumours. *International Journal of Cancer*, **22**, 413–421.

Rutanen, E.M. and Seppala, M. (1978) The hCG-beta subunit radioimmunoassay in nontrophoblastic gynecologic tumors. *Cancer*, **41**, 692–696.

Ryan, R.J., Keutmann, H.T., Charlesworth, M.C., McCormick, D.J., Milius, R.P., Calvo, F.O. and Vutyavanich, T. (1987) Structure-function relationships of gonadotropins. *Recent Progress in Hormone Research*, **43**, 383–429.

Ryan, R.J., Charlesworth, M.C., McCormick, D.J., Morris, J.C., Milius, R., Begert, E. and

Keutmann, H.T. (1988) Some structural and functional features of the glycoprotein hormones, in *Placental Protein Hormones*, (eds M. Mochizuki and R. Hussa), Elsevier, Amsterdam, pp. 111–122.

Rzasa, P.J., Caride, V.J. and Prokop, E.K. (1984) Discordant inter-kit results in the radioimmunoassay for choriogonadotropin in serum. *Clinical Chemistry*, **30**, 1240–1243.

Sagen, N., Nilse, S.T., Kimm, H.C., Koller, O. and Bergsjo, P. (1984) The predictive value of total estriol, HPL and HB on perinatal outcome in severe pre-eclampsia. *Acta Obstetrica et Gynecologica Scandinavica*, **63**, 603–608.

Said, M.E., Campbell, D.M., Azzam, M.E. and MacGillivray, I. (1984) Beta-human chorionic gonadotrophin levels before and after development of pre-eclampsia. *British Journal of Obstetrics and Gynaecology*, **91**, 772–775.

Sairam, M.R. and Bhargavi, G.N. (1985) A role for glycosylation of the α-subunit in transduction of biological signal in glycoprotein hormones. *Science*, **229**, 65–67.

Sakbun, V., Ali, S., Greenwood, F.C. and Bryant-Greenwood, G.D. (1990) Human relaxin in the amnion, chorion, decidua parietalis, basal plate and placental trophoblast by immunohistochemistry and Northern analysis. *Journal of Clinical Endocrinology and Metabolism*, **70**, 508–514.

Salem, H.T., Lee, J.N., Seppala, M., Vaara, L., Aula, P., Al-Ani, A.T.M. and Chard, T. (1981) Measurement of placental protein 5, placental lactogen and pregnancy-specific glycoprotein in mid-trimester as a predictor of outcome of pregnancy. *British Journal of Obstetrics and Gynaecology*, **88**, 371–374.

Salem, H.T., Shaaban, M.M., Ghaneimah, S.A., Seppala, M. and Chard, T. (1983) Circulating levels of placental proteins, placental protein 5 (PP5), placental lactogen and Schwangerschaftsprotein 1 (SP1), in antepartum eclampsia. *British Journal of Obstetrics and Gynaecology*, **90**, 618–622.

Salem, H.T., Menabawey, M., Seppala, M., Shaaban, M.M. and Chard, T. (1984a) Human seminal plasma contains a wide range of trophoblast specific proteins. *Placenta*, **5**, 413–418.

Salem, H.T., Ghaneimah, S.A., Shaaban, M.M. and Chard, T. (1984b) Prognostic value of biochemical tests in the assessment of fetal outcome in threatened abortion. *British Journal of Obstetrics and Gynaecology*, **91**, 382–385.

Saller, B., Clara, R., Spottl, G., Siddle, K. and Mann, K. (1990) Testicular cancer secretes intact choriogonadotropin (hCG) and its free β-subunit: evidence that hCG(+hCG-B) assays are the most reliable in diagnosis and follow-up. *Clinical Chemistry*, **36**, 234–239.

Samaan, N., Yen, S.C.C. and Friesen, H.G. (1966) Serum placental lactogen levels during pregnancy and in trophoblastic disease (abstract). *Journal of Clinical Endocrinology*, **26**, 1303–1308.

Saxena, B.B., Goldstein, D.P., Emerson, K. and Selenkow, H.A. (1968) Serum placental lactogen levels in patients with molar pregnancy and trophoblastic tumors (abstract). *American Journal of Obstetrics and Gynecology*, **102**, 115–121.

Saxena, B.B., Emerson, K. and Selenkow, H.A. (1969) Serum placental lactogen (HPL) levels as an index of placental function (abstract). *New England Journal of Medicine*, **281**, 225–231.

Schneider, A.B., Kowalski, K., Buckman, G. and Sherwood, L.M. (1977) Dimeric human placental lactogen, immunological and biological activity (abstract). *Biochimica et Biophysica Acta*, **493**, 69–77.

Schoknecht, P.A., Nobrega, S.N., Petterson, J.A., Ehrhardt, R.A., Slepetis, R. and Bell, A.W. (1991) Relations between maternal and fetal plasma concentrations of placental lactogen and placental and fetal weights in well-fed ewes. *Journal of Animal Science*, **69**, 1059–1063.

Schreyer, P., Zer, Y., Ariely, S., Herman, A. and Caspi, E. (1989) Influence of ritodrine on plasma steroids and human placental lactogen levels in third trimester pregnancies. *Gynecologic and Obstetric Investigation*, **27**, 10–13.

Schuette, J., Niederle, N., Scheulen, M.E., Seeber, S. and Schmidt, C.G. (1985) Chemotherapy of metastatic seminoma. *British Journal of Cancer*, **51**, 467–472.

Schwarz, S., Berger, P. and Wick, G. (1985) Epitope-selective, monoclonal-antibody-based immunoradiometric assays of predictable specificity for differential measurement of choriogonadotropin and its subunits. *Clinical Chemistry*, **31**, 1322–1328.

Sciarra, J.J., Sherwood, L.M., Varma, A.A. and Lundberg, W.B. (1968) Human placental lactogen (HPL) and placental weight (abstract). *American Journal of Obstetrics and Gynecology*, **101**, 413–416.

Selby, M.J., Barta, A., Baxter, J.D., Bell, G.I. and Eberhardt, N.L. (1984) Analysis of a major human chorionic somatomammotropin gene: evidence for two functional promoter elements

(abstract). *Journal of Biological Chemistry*, **259**, 13131–13138.

Selenkow, H.A., Varma, K., Younger, D., White, P. and Emerson, K. (1969) Patterns of serum immunoreactive human placental lactogen (IR-HPL) and chorionic gonadotropin (IR-HCG) in diabetic pregnancy (abstract). *Diabetes*, **20**, 696–706.

Selvanayagam, C.S., Tsait, S.Y., Tsait, M.J., Selvanayagam, P. and Saunders, G.F. (1984) Multiple origins of transcription of the human placental lactogen genes. *Journal of Biological Chemistry*, **259**, 4642–4646.

Seppala, M., Lehtovirta, P. and Rutanen, E.M. (1978) Detection of chorionic gonadotrophin like activity in infertile cycles. *Acta Endocrinologica*, **88**, 164–168.

Seppala, M., Ranta, T., Tontti, K., Stenman, U.H. and Chard, T. (1980) Use of a rapid hCG-beta subunit radioimmunoassay in acute gynaecological emergencies. *Lancet*, **1**, 165–166.

Seppala, M., Iino, K. and Rutanen, E.V. (1986) Placental proteins in oncology. *Clinics in Obstetrics and Gynaecology*, **14**, 593–610.

Shapiro, H.A. and Swarenstein, H.A. (1934) A rapid test for pregnancy on Xenopus laevis. *Nature*, **133**, 762.

Sharp, N.C, Anthony, F., Miller, J.F. and Masson, G.M. (1986) Early conceptual loss in subfertile patients. *British Journal of Obstetrics and Gynaecology*, **93**, 1072–1077.

Sherman, G.B., Wolfe, M.W., Farmerie, T.A., Clay, C.M., Threadgill, D.S., Sharp, D.C. and Nilson, J.H. (1992) A single gene encoded the β-subunits of equine luteinizing hormone and chorionic gonadotropin. *Molecular Endocrinology*, **6**, 951–959.

Sherwood, L.M., Burstein, Y. and Schecter, T. (1979) Primary structure of the NH_2 terminal extra piece of the precursor to human placental lactogen (abstract). *Proceedings of the National Academy of Sciences of the USA*, **76**, 3819–3823.

Shida, M.M., Jackson-Grusby, L.L., Ross, S.R. and Linzer, D.I. (1992) Placental-specific expression from the mouse placental lactogen II gene promoter. *Proceedings of the National Academy of Sciences of the USA*, **89**, 3864–3868.

Shinde, S.R., Adil, M.A., Sheth, A.R., Kjoppikar, M.G. and Sheth, N.A. (1981) Ectopic human placental lactogen and beta-human chorionic gonadotropin in gastric fluid of patients with malignant and non-malignant conditions of the stomach (abstract). *Oncology*, **38**, 277–280.

Shome, B. and Parlow, A.F. (1973) The primary structure of the hormone specific beta subunit of human pituitary luteinizing hormone (hLH). *Journal of Clinical Endocrinology and Metabolism*, **36**, 618–621.

Shome, B. and Parlow, A.F. (1977) The entire linear amino acid sequence (abstract). *Journal of Clinical Endocrinology*, **45**, 1112–1115.

Shu-Rong, Z., Bremme, K., Eneroth, P. and Nordberg, A. (1982) The regulation of *in vitro* placental release of human chorionic gonadotropin, placental lactogen and prolactin: effects of an adrenergic beta-receptor agonist and antagonist. *American Journal of Obstetrics and Gynecology*, **143**, 444–450.

Sideri, M., De Virgiliis, G., Guidobono, F., Borgese, N., Sereni, L.P., Nicolini, U. and Remotti, G. (1983) Immunologically undetectable human placental lactogen in normal pregnancy. Case report. *British Journal of Obstetrics and Gynaecology*, **90**, 771–773.

Siler-Khodr, T.M., Khodr, G.S., Vickery, B.H. and Nestor, J.J. (1983) Inhibition of hCG, alpha hCG and progesterone release from human placental tissue *in vitro* by a GnRH antagonist. *Life Sciences*, **32**, 2741–2745.

Siler-Khodr, T.M., Khodr, G.S., Valenzuela, G. and Rhode, J. (1986) Gonadotrophin-releasing hormone effects on placental hormones during gestation. I. Alpha-human chorionic gonadotropin, human chorionic gonadotropin and human chorionic somatomammotropin. *Biology of Reproduction*, **34**, 245–254.

Simon, P., Decoster, C., Brocas, H., Schwers, J. and Vassart, G. (1986) Absence of human chorionic somatomammotropin during pregnancy associated with two types of gene deletion. *Human Genetics*, **74**, 235–238.

Soares, M.J. and Talamantes, F. (1983) Genetic and litter size effects on serum placental lactogen in the mouse. *Biology of Reproduction*, **28**, 165–171.

Soares, M.J. and Talamantes, F. (1985) Placental lactogen secretion in the mouse: *in vitro* responses and ovarian and hormonal influences. *Journal of Experimental Zoology*, **234**, 97–102.

Soares, M.J., Colosi, P. and Talamantes, F. (1982) The development and characterisation of a homologous radioimmunoassay for mouse placental lactogen. *Endocrinology*, **11**, 668–676.

Soler, N.G., Nicholson, H.O. and Malins, J.M. (1975) Serial determinations of human placental

lactogen in the management of diabetic pregnancy. *Lancet*, **2**, 54–57.

Soma, H., Kikuchi, K., Takayama, M., Saegusa, H., Saito, T., Kashiwagi, H. and Sayama, S. (1981) Concentrations of SP1 and beta-hCG in serum and cerebrospinal fluid and concentrations of hCG in urine in patients with trophoblastic tumour. *Archives of Gynecology*, **230**, 321–327.

Southard, J.N. and Talamantes, F. (1989) High molecular weight forms of placental lactogen: evidence for lactogen–macroglobulin complexes in rodents and humans. *Endocrinology*, **125**, 791–800.

Southard, J.N., Thordarson, G. and Talamantes, F. (1986) Purification and partial characterization of hamster placental lactogen. *Endocrinology*, **119**, 508–514.

Southard, J.N., Campbell, G.T. and Talamantes, F. (1987) Hamster placental lactogens: gestational profiles and high molecular weight forms. *Endocrinology*, **121**, 900–906.

Sowers, J.R., Pekary, A.E., Hershman, J.M., Kanter, M. and Distefano, J.J. (1979) Metabolism of exogenous human chorionic gonadotropin in men. *Journal of Endocrinology*, **80**, 83–89.

Spellacy, W.N., Carlson, K.L. and Birk, S.A. (1966) Dynamics of human placental lactogen. *American Journal of Obstetrics and Gynecology*, **96**, 1164–1173.

Spellacy, W.N., Buhi, W.C., Schram, J.D. and Birk, S.A. (1971) Control of human chorionic somatomammotrophin levels during pregnancy. *Obstetrics and Gynecology*, **37**, 567–572.

Spellacy, W.N., Buhi, W.C. and Birk, S.A. (1975) The effectiveness of human placental lactogen measurements as an adjunct in decreasing perinatal deaths. *American Journal of Obstetrics and Gynecology*, **121**, 835–844.

Spellacy, W.N., Buhi, W.C. and Birk, S.A. (1977) The effect of smoking on serum human placental lactogen levels. *American Journal of Obstetrics and Gynecology*, **127**, 232–234.

Spellacy, W.N., Cruz, A., Kalra, P.S., Kellner, K.R., Quinlan, R.W. and Buhi, W.C. (1979) Oxytocin challenge test results compared with simultaneously studied serum human placental lactogen and free estriol levels in high-risk pregnant women. *American Journal of Obstetrics and Gynecology*, **135**, 917–923.

Spencer, K., Coombes, E.J., Mallard, A.S. and Milford-Ward, A. (1992) Free beta human choriogonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers. *Annals of Clinical Biochemistry*, **29**, 506–518.

Spies, H.G. and Chappel, S.C. (1984) Mammals: non-human primates, in *Marshall's Physiology of Reproduction*, Vol. 4, 4th edn (ed. G.E. Lamming), Churchill Livingstone, Edinburgh, pp. 659–712.

Sprague, A.D., Duhring, J.L., Moser, R.J. and Hollingsworth, D. (1973) Maternal and fetal levels of HCS in preeclampsia. *Obstetrics and Gynecology*, **41**, 770–773.

Stanhope, C.R., Smith, J.P., Brittan, J.C. and Crosley, P.K. (1979) Serial determination of marker substances in ovarian cancer. *Gynecological Oncology*, **8**, 284–287.

Stenman, U.H., Bidart, J.M., Birken, S., Mann, K., Nisula, B. and O'Connor, J. (1993) Standardization of protein immunoprocedures. Choriogonadotropin (CG). *Scandinavian Journal of Clinical Laboratory Investigation*, **53**, Suppl.216, 42–79.

Stevens, V.C. (1975) Female contraception by immunization with hCG – prospects and status, in *Immunization with Hormones in Reproductive Research*, (ed. E. Nieschlag), North-Holland Publishing, Amsterdam, pp. 217–222.

Stevens, V.C. (1976) Antifertility effects of immunizations with intact, subunits and fragments of hCG, in *Physiological Effects of Immunity against Reproductive Hormones*, (eds R.G. Edwards and M.H. Johnson), Cambridge University Press, Cambridge, pp. 249–264.

Stevens, V.C. (1986) Use of synthetic peptides as immunogens for developing a vaccine against human chorionic gonadotropin, in *Synthetic Peptides as Antigens*, Wiley, Chichester, pp. 200–225.

Stevens, V.C. and Crystle, G.D. (1973) Effects of immunization with hapten-coupled hCG on the human menstrual cycle. *Obstetrics and Gynecology*, **42**, 484–495.

Stevens, V.C., Powell, T.E., Lee, A.C. and Griffin, D. (1981) Antifertility effects of immunization on female baboons with C-terminal peptides of the β-subunit of human chorionic gonadotropin. *Fertility and Sterility*, **36**, 98–105.

Stewart, F. and Maher, J.K. (1991) Analysis of horse and donkey gonadotrophin genes using southern blotting and DNA hybridization techniques. *Journal of Reproduction and Fertility*, **44**, 19–25.

Stewart, M.O., Whittaker, P.G., Persson, B.,

Hanson, U. and Lind, T. (1989) A longitudinal study of circulating progesterone, oestradiol, hCG and hPL during pregnancy in type 1 diabetic mothers. *British Journal of Obstetrics and Gynaecology*, **96**, 415–423.

Stockell Hartree, A. (1989) Multiple forms of pituitary and placental gonadotropins, in *Oxford Reviews of Reproductive Biology*, (ed. S.R. Milligan), Oxford University Press, Oxford, pp. 147–165.

Storring, P.O., Gaines-Das, R.E. and Bangham, D.R. (1980) International reference preparation of human chorionic gonadotropin for immunoassay: potency estimates in various bioassay and protein binding assay systems; and international reference preparations of the α and β subunits of human chorionic gonadotropin for immunoassay. *Journal of Endocrinology*, **84**, 295–310.

Suganuma, N., Matzuk, M.M. and Boime, I. (1989) Elimination of disulphide bonds affects assembly and secretion of the human chorionic gonadotropin β-subunit. *Journal of Biological Chemistry*, **264**, 19302–19307.

Suwa, S. and Friesen, H. (1969a) Biosynthesis of human placental proteins and human placental lactogen (hPL) *in vitro*. I. Identification of ^{3}H-labelled hPL. *Endocrinology*, **85**, 1028–1036.

Suwa, S. and Friesen, H. (1969b) Biosynthesis of human placental proteins and human placental lactogen (hPL) *in vitro*. II. Dynamic studies of normal term placentas. *Endocrinology*, **85**, 1037–1045.

Swenne, I., Hill, D.J., Strain, A.J. and Milner, R.D.G. (1987) Effects of human placental lactogen and growth hormone on the production of insulin and somatomedin C/insulin-like growth factor I by human fetal pancreas in tissue culture. *Journal of Endocrinology*, **113**, 297–303.

Taketani, Y., Taga, M., Minaguchi, H., Mizuno, M. and Sakamoto, S. (1984) Elevated concentrations of prolactin, human growth hormone and human placental lactogen in cord sera obtained for light-for-date babies near term. *Asia–Oceania Journal of Obstetrics and Gynaecology*, **10**, 235–240.

Talmadge, K., Vamvakopoulos, N.C. and Fiddes, J.C. (1984a) Evolution of the genes for the beta subunit of human chorionic gonadotropin and luteinizing hormone. *Nature*, **307**, 37–40.

Talmadge, K., Boorstein, W.R., Vamvakopoulos, N.C., Gething, M.J. and Fiddes, J.C. (1984b) Only three of the seven human chorionic gonadotrophin β-subunit genes can be expressed in the placenta. *Nucleic Acids Research*, **12**, 8145–8436.

Tatra, G., Tempfer, H. and Placheta, P. (1977) Influence of amino acid administration on serum concentrations of pregnancy-specific proteins SP1 and hPL during last trimester of pregnancy. *European Journal of Gynecology and Reproductive Biology*, **7**, 29–32.

Tatra, G., Tempfer, H. and Gruber, W. (1984) Influence of 16-beta-methylprednisone on serum concentrations of pregnancy specific protein SP1 and hPL during the last trimester of pregnancy. *European Journal of Gynecology and Reproductive Biology*, **6**, 49–52.

Taylor, M.J., Jenkin, G., Robinson, J.S., Thorburn, G.D., Friesen, H. and Chan, J.S.D. (1980) Concentrations of placental lactogen in chronically catheterized ewes and fetuses in late pregnancy. *Journal of Endocrinology*, **85**, 27–34.

Taylor, M.J., Jenkin, G., Robinson, J.S. and Thorburn, G.D. (1982) Regulation of ovine placental lactogen: lack of correlation with progesterone secretion. *Journal of Endocrinology*, **95**, 275–279.

Taylor, M.J., Robinson, J.S., Jenkin, G. and Thorburn, G.D. (1983a) Ovine placental lactogen concentrations during premature delivery induced by infusion of dexamethasone or adrenocorticotrophin to the fetus. *Journal of Endocrinology*, **98**, 197–200.

Taylor, M.J., McMillen, C., Jenkin, G., Robinson, S. and Thorburn, G.D. (1983b) The regulation of ovine placental lactogen the role of the fetal hypothalamic–pituitary axis. *Journal of Developmental Physiology*, **5**, 251–258.

Tepper, M.A. and Roberts, J.L. (1984) Evidence for only one β-luteinizing hormone and no β-chorionic gonadotropin gene in the rat. *Endocrinology*, **115**, 385–391.

Thanavale, Y.Y., Hay, F.C. and Stevens, V.C. (1978) Immunological control of fertility: measurement of the affinity of antibodies to human chorionic gonadotropin. *Clinical and Experimental Immunology*, **33**, 403–409.

Thomas, G.M. (1985) Controversies in the management of testicular seminoma. *Cancer*, **55**, 2296–2302.

Thomson, M.A.R., Duncan, R.O. and Cunningham, P. (1988) The value of serum human placental lactogen and Schwangerschaftsprotein 1 to determine gestation in an

ante-natal population. *Human Reproduction*, **3**, 463–465.

Thordarson, G., Villalobos, R., Colosi, P., Southard, J., Ogren, L. and Talamantes, F. (1986) Lactogenic response of cultured mouse mammary epithelial cells to mouse placental lactogen. *Journal of Endocrinology*, **109**, 263–274.

Thordarson, G., McDowell, G.H., Smith, S.V., Iley, S. and Forsyth, I.A. (1987) Effects of continuous intravenous infusion of an ovine placental extract enriched in placental lactogen on plasma hormones, metabolites, and metabolite biokinetics in non-pregnant sheep. *Journal of Endocrinology*, **113**, 277–283.

Tominaga, N., Sakakibaro, R. and Yokoo, Y. (1989) Existence of associated, non- associated and oligomeric forms of human chorionic gonadotropin subunits in placental extracts. *Journal of Biochemistry (Tokyo)*, **105**, 992–997

Tonkowicz, P.A. and Voogt, J.L. (1984) Ovarian and fetal control of rat placental lactogen and prolactin secretion at midpregnancy. *Endocrinology*, **114**, 254–259.

Tormey, D.C., Waalkes, T.P., Ahmann, D., Gehrke, C.W., Zumwatt, R.W., Snyder, J. and Hansen, H. (1975) Biological markers in breast carcinoma. I. Incidence of abnormalities of CEA, hCG, 3 polyamines and 3 minor nucleotides. *Cancer*, **35**, 1095–1100.

Trapp, M., Kato, K., Bohnet, H.G., Gerhard, I., Weise, H.C. and Leidenberger, F. (1986) Human placental lactogen and unconjugated estriol concentrations in twin pregnancy: monitoring of fetal development in intrauterine growth retardation and single intrauterine death. *American Journal of Obstetrics and Gynecology*, **155**, 1027–1031.

Tsong, Y.Y., Chang, C.C. and Nash, H.A. (1985) Formulation of a potent anti pregnancy vaccine based on the β-subunit of human chorionic gonadotropin (β-hCG). I. Alternative macromolecular carriers. *Journal of Reproductive Immunology*, **7**, 139–149.

Tsunasawara, S., Liu, W.K., Burleigh, B.D. and Ward, D.N. (1977) Studies of disulphide bond location in ovine lutropin β-subunit. *Biochimica et Biophysica Acta*, **492**, 340–356.

Tullner, W. and Hertz, R. (1966) Chorionic gonadotropin levels in the rhesus monkey during early pregnancy. *Endocrinology*, **78**, 204–207.

Tulsky, A.S. and Koff, A.K. (1957) Some observations on the role of the corpus luteum in early pregnancy. *Fertility and Sterility*, **8**, 181–186.

Tyson, J.E., Rabinowitz, D., Merimee, T.J. and Friesen, H. (1969) Response of plasma insulin and human growth hormone to arginine in pregnant and post partum females. *American Journal of Obstetrics and Gynecology*, **103**, 313–319.

Ursell, W., Brudenell, M. and Chard, T. (1973) Placental lactogen levels in diabetic pregnancy. *British Medical Journal*, **1**, 80–82.

Vaitukaitis, J.L. (1973) Immunological and physical characterization of human chorionic gonadotropin (hCG) secreted by tumors. *Journal of Clinical Endocrinology and Metabolism*, **37**, 505–514.

Vaitukaitis, J.L. (1985) Radioimmunoassay of human choriogonadotropin. *Clinical Chemistry*, **31**, 1749–1754.

Vaitukaitis, J. and Ebersole, E.R. (1976) Evidence for altered synthesis of human chorionic gonadotropin in gestational trophoblastic tumours. *Journal of Clinical Endocrinology and Metabolism*, **42**, 1048–1055.

Vaitukaitis, J.L., Braunstein, G.D. and Ross, G.T. (1972) A RIA which specifically measures hCG in the presence of LH. *American Journal of Obstetrics and Gynecology*, **113**, 751–757.

Valkirs, G.E. and Barton, R. (1985) Immunoconcentration – a new format for solid-phase immunoassays. *Clinical Chemistry*, **31**, 1427–1431.

Van Hall, E.V., Vaitukaitis, J.L. and Ross, G.T. (1971) Immunological and biological activity of hCG following progressive desialation. *Endocrinology*, **88**, 456–464.

Virgin, J.B., Silver, B.J., Thomason, A.R. and Nilson, J.H. (1985) The gene for the β-subunit of bovine luteinizing hormone encodes a gonadotropin mRNA with an unusually short 5′-untranslated region. *Journal of Biological Chemistry*, **260**, 7072–7077.

Voogt, J., Robertson, M. and Friesen, H. (1982) Inverse relationship of prolactin and rat placental lactogen during pregnancy. *Biology of Reproduction*, **26**, 800–805.

Voogt, J.L., Pakrasi, P.L., Johnson, D.C. and Dey, S.K. (1985) Evidence for pituitary regulation of placental lactogen in the rat. *Journal of Endocrinology*, **107**, 121–126.

Wald, N.J., Cuckle, H.S., Densem, J.W., Nanchahal, K., Royston, P., Chard, T., Haddow, J.E., Knight, G.J., Palomaki, G.E. and Canick, J.A. (1988) Maternal serum screening for Down's syndrome in early pregnancy. *British Medical Journal*, **297**, 883–887.

Walker, W.H., Fitzpatrick, S.L. and Saunders,

G.F. (1990) Human placental lactogen transcriptional enhancer. *Journal of Biological Chemistry*, **265**, 12940–12948.

Walsh, S.W., Wolf, R.C., Meyer, R.K., Aubert, M.L. and Friesen, H.G. (1977) Chorionic gonadotropin, chorionic somatomammotropin, and prolactin in the uterine vein and peripheral plasma of pregnant rhesus monkeys. *Endocrinology*, **100**, 851–855.

Wang, H., Segal, S.J. and Koide, S.S. (1988) Purification and characterization of an incompletely glycosylated form of human chorionic gonadotropin from human placenta. *Endocrinology*, **123**, 795–803.

Wang, Y., Schwartz, P.E., Chambers, J.T. and Cole, L.A. (1988) Urinary gonadotrophin fragment (UGF) in cancers of the reproductive tract. II. Initial series studies. *Gynecological Oncology*, **31**, 91–100.

Ward, R.H.T., Letchworth, A.T., Niven, P.A.R. and Chard, T. (1974) Placental lactogen levels in rhesus isoimmunization. *British Medical Journal*, **1**, 347–349.

Ward, R.H.T., Whyley, G.A., Fairweather, D.V.I., Allen, E.I. and Chard, T. (1977) A comparative study of plasma 17β-oestradiol, progesterone, placental lactogen and chorionic gonadotrophin in abortion induced with intra-amniotic prostaglandin $F_{2\alpha}$. *British Journal of Obstetrics and Gynaecology*, **84**, 363–369.

Warren, W.C., Liang, R., Krivi, G.G., Siegel, N.R. and Anthony, R.V. (1990) Purification and structural characterization of ovine placental lactogen. *Journal of Endocrinology*, **126**, 141–149.

Waters, M.J., Oddy, V.H., McCloghry, C.E., Gluckman, P.D., Diplock, R., Owens, P.C. and Brinsmead, M.W. (1985) An examination of the proposed roles of placental lactogen in the ewe by means of antibody neutralization. *Journal of Endocrinology*, 106, 377–386.

Wehmann, R.E. and Nisula, B.C. (1980a) Renal clearance rates of the subunits of human chorionic gonadotropin in man. *Journal of Clinical Endocrinology and Metabolism*, **50**, 101–105.

Wehmann, R.E. and Nisula, B.C. (1980b) Characterization of a discrete degradation product of the human chorionic gonadotropin β-subunit in humans. *Journal of Clinical Endocrinology and Metabolism*, **51**, 101–105.

Wehmann, R.E. and Nisula, B.C. (1981) The metabolic and renal clearance rates of purified human chorionic gonadotropin. *Journal of Clinical Investigation*, **68**, 186–194.

Wehmann, R.E., Blithe, D.L., Akar, A.H. and Nisula, B.C. (1987) β-core fragments are contaminants of the World Health Organization Reference Preparations of human choriogonadotropin and its α-subunit. *Journal of Endocrinology*, **117**, 147–152.

Wehmann, R.E., Blithe, D.L., Flack, M.R. and Nisula, B.C. (1989) Metabolic clearance rate and urinary clearance of purified beta-core. *Journal of Clinical Endocrinology and Metabolism*, **69**, 510–517.

Wehmann, R.E., Blithe, D.L., Akar, A.H. and Nisula, B.C. (1990) Disparity between β-core levels in pregnancy urine and serum: implications for the origin of urinary β-core. *Journal of Clinical Endocrinology and Metabolism*, **70**, 371–378.

Westergaard, J.C., Teisner, B., Grudzinskas, J.G. and Chard, T. (1985a) Placental function studies in low birth weight infants with and without dysmaturity. *Obstetrics and Gynaecology*, **65**, 316–318.

Westergaard, J.G., Teisner, B., Grudzinskas, J.G. and Chard, T. (1985b) Single measurements of chorionic gonadotropin and Schwangerschafts protein for assessing gestational age and predicting day of delivery. *Journal of Reproductive Medicine*, **30**, 57–60.

Westergaard, J.G., Teisner, B., Grudzinskas, J.G. and Chard, T. (1985c) Pregnancy associated plasma protein A (PAPP-A) in preovulatory, nonovulatory healthy and atretic human ovarian follicles during the natural cycle. *Annals of the New York Academy of Science*, **442**, 205–211.

White, J.W., Sobnosky, M., Rogers, B.L., Walker, W.H. and Saunders, G.F. (1993) Nucleotide sequence of a transcriptional enhancer located 2.2 kb 3′ of a human placental lactogen-encoding gene. *Gene*, **84**, 521–522.

Whitfield, G.K. and Kourides, I.A. (1985) Expression of chorionic gonadotropin alpha- and beta-genes in normal and neoplastic human tissues: relationship to deoxyribonucleic acid structure. *Endocrinology*, **117**, 231–236.

Whittaker, P.G., Taylor, A. and Lind, T. (1983) Unsuspected pregnancy loss in healthy women. *Lancet*, **1**, 1126–1127.

WHO (1985) Special programme of research, development and research training in human reproduction. *Fourteenth Annual Report*, 53–61.

Wide, L. and Gemzell, C.A. (1960) An immunological pregnancy test. *Acta Endocrinology*, **35**, 261–267.

Wide, L. and Hobson, B. (1974) Relationship between the sex of the fetus and the amount of human chorionic gonadotropin in placentae from the 10th – 20th week of pregnancy. *Journal of Endocrinology*, **61**, 75–81.

Wilcox, A.J., Weinburg, C.R., Wehmann, R.E., Armstrong, E.G., Canfield, R.E. and Nisula, B.C. (1985) Measuring early pregnancy loss: laboratory and field methods. *Fertility and Sterility*, **44**, 366–374.

Willey, K.P. and Leidenberger, F. (1989) Functionally distinct agonist and receptor-binding regions in human chorionic gonadotropin. *Journal of Biological Chemistry*, **264**, 19716–19729.

Wilson, C.A., Leigh, A.J. and Chapman, A.J. (1990) Gonadotropin glycosylation and function. *Journal of Endocrinology*, **125**, 3–14.

Wilson, E.A. and Jawad, M.J. (1982) Stimulation of human chorionic gonadotropin secretion by glucocorticoids. *American Journal of Obstetrics and Gynecology*, **142**, 344–348.

Wilson, E.A., Jawad, M.J. and Dickson, L.H. (1980) Suppression of human chorionic gonadotropin by progestational steroids. *American Journal of Obstetrics and Gynecology*, **138**, 708–713.

Wilson, E.A., Jawad, M.J. and Powell, D.E. (1984a) Effect of estradiol and progesterone on human chorionic gonadotropin secretion *in vitro*. *American Journal of Obstetrics and Gynecology*, **149**, 143–148.

Wilson, E.A., Jawad, M.J. and Vernon, M.W. (1984b) Effect of epidermal growth factor on hormone secretion by term placenta in organ culture. *American Journal of Obstetrics and Gynecology*, **149**, 579–580.

Wiltberger, P.B. and Miller, D.F. (1948) The male frog, Rana pipiens, as a new test animal for early pregnancy. *Science*, **107**, 198.

Wooding, F.B., Morgan, G., Forsyth, I.A., Butcher, G., Hutchings, A., Billingsley, S.A. and Gluckman, P.D. (1992) Light and electron microscopic studies of cellular localization of oPL with monoclonal and polyclonal antibodies. *Journal of Histochemistry and Cytochemistry*, **40**, 1001–1009.

Yamaguchi, M., Endo, H., Thordarson, G., Ogren, L. and Talamantes, F. (1992) Modulation of mouse placental lactogen-I secretion *in vitro*: effects of progesterone and mouse placental lactogen-II. *Endocrinology*, **130**, 2897–2905.

Yamini, G., Chard, T. and Blake, M.E. (1972) Some biological effects of antisera to human placental lactogen in rodents. *Journal of Endocrinology*, **55**, 29.

Yazaki, K., Yazaki, C., Wakabayashi, K. and Igarashi, M. (1980) Isoelectric heterogeneity of human chorionic gonadotropin: presence of choriocarcinoma-specific components. *American Journal of Obstetrics and Gynecology*, **138**, 189–194.

Ylikorkala, O. and Pennanen, S. (1973) Human placental lactogen (hPL) levels in maternal serum during abortion induced by intra-and extra amniotic injection of prostaglandin F2-alpha. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 927–931.

Yogev, L., Gibber, J.R. and Terkel, J. (1983) Failure of rat placenta to inhibit rat prolactin secretion by ectopic pituitary gland. *Journal of Endocrinology*, **97**, 91–95.

Yoshimi, T., Strott, C., Marshall, J. and Lipsett, M. (1969) Corpus luteum function in early pregnancy. *Journal of Clinical Endocrinology and Metabolism*, **29**, 225–230.

Yoshimoto, Y., Wolfsen, A.R., Hirose, F. and Odell, W.D. (1979) Human chorionic gonadotropin-like material: presence in normal human tissues. *American Journal of Obstetrics and Gynecology*, **134**, 729–733.

Yovich, J.L., Wilcox, D.L., Grudzinskas, H.G. and Bolton, A.E. (1986) The prognostic value of hCG, PAPP-A, oestradiol-17 beta and progesterone in early human pregnancy. *Australia and New Zealand Journal of Obstetrics and Gynaecology*, **26**, 59–64.

Zeitler, P., Markoff, E. and Handwerger, S. (1983) Characterization of the synthesis and release of human placental lactogen and human chorionic gonadotropin by an enriched population of dispersed placental cells. *Journal of Clinical Endocrinology and Metabolism*, **57**, 812–818.

Zondek, B. (1930) Schwangerschafts reaktion aus dem harn bei Mensch und Tier. *Klinische Wochenschrift*, **9**, 2285–2289.

NORMAL AND ABNORMAL EMBRYO–FETAL DEVELOPMENT IN MAMMALS

6

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6.1 INTRODUCTION

In this chapter we examine the first interactions between embryo and mother at the start of pregnancy: the biochemical messages that pass between them and the morphological links that are established. We consider the function of these biochemical signals in facilitating the process of implantation and the support of the corpus luteum of pregnancy. We review in some detail the growth and development of the embryo, the genetic basis for sexual differentiation and the aetiology of embryonic and fetal abnormality.

The past few years have seen considerable advances in our understanding of the establishment of pregnancy. Yet, because of the difficulties of accessibility *in situ* and the ethical constraints involved, it is not feasible to examine in much detail the process of implantation and corpus luteum rescue at a local, cellular level. This is particularly true for the primates, including man. The huge increase in research on pre-implantation development in man, associated with *in vitro* fertilization and related techniques, (Edwards, 1985) has shown the specificity of mechanisms operating in the primate and also our ignorance of the underlying cellular and molecular processes. Examples are the nutrient requirements for the embryo during its development from fertilization to implantation, the limits to potential in the regulation of blastomere, inner cell mass and trophoblast differentiation, the embryo maternal dialogue that results in the implantation of the fetal allograft, the genetic or other causes of abnormalities causing death or deformity of the embryo, and the flexibility possible in the embryo's growth rates and endocrine support.

Our main consideration here is the primates, including man. Where our knowledge on primates is insufficient, we develop our arguments with knowledge derived from other species. Throughout we refer to recent reviews in non-primate biology in order to provide leads for those readers who wish to pursue these questions further, but normal and abnormal embryo and fetal development in primates is our principal theme in this review.

In section 6.2 the two questions are:

1. What are the biochemical signals between embryo and mother at the start of pregnancy in primates?
2. How do these signals enhance (a) implantation and invasion of trophoblast, (b) nutritional and immunological security for the fetal allograft, (c) transformation of the corpus luteum (CL) to support pregnancy and (d) the differentiation of the early embryo through programmed gene activation?

During the past 15 years this field of research has moved from an emphasis on whole animals and systems towards a cellular and now towards a molecular level. We and others have developed *in vivo* and *in vitro* systems to explore cell–cell recognition and molecular mechanisms while retaining as far as possible the physiological validation of these findings in the whole animal. Where feasible, we have run parallel studies with human tissues, but the key questions stated above cannot be approached in detail on an experimental level using the human directly. In addition, there are fundamental differences between the primate and non-primate systems that ensure implantation and corpus luteum function. Consequently, while a vast amount of knowledge and technique developed on non-primate species has enabled studies to be carried out in primates, the results cannot often be interpreted across species. Indeed, there is considerable variation in the ways in which these processes have evolved even between primate species. Wimsatt (1975) has provided an excellent review of some comparative aspects of implantation. (See also Chapter 4.)

In section 6.3 we explore the development

of the embryo and the signals that it produces during the peri-implantation period. With the exception of chorionic gonadotrophin (CG) there is, as yet, little clear evidence for the physiological role of embryo-derived signals in primates, but there are several candidates whose function can be tested with the culture systems and molecular techniques now available. An example is the polymerase chain reaction (PCR), which allows amplification of message from small amounts of embryonic tissue. We do not discuss the biology of trophoblast or the placental proteins that are secreted well after implantation. There is a large amount of literature which can be followed up outside the scope of this chapter (for example: Schlafke and Enders, 1975; Loke and Whyte, 1983; Chapman *et al.*, 1988; and Chapter 5 of this volume).

Section 6.3 concerns the functions of embryonic signals at the site of implantation and the corpus luteum, together with the effects of immunological inhibition. We consider also the direct or indirect interactions and their timing between the embryo, corpus luteum and the hypothalamus. The rates of development of the embryo and fetus in a variety of mammalian species are considered in section 6.4. The constitutive and environmental factors which define the normal range of prenatal growth and birth weight, as well as the constraining influences that can result in embryo/fetal loss during pregnancy, have been observed in all mammals studied, although there are marked differences between species in the incidence and timing of prenatal death. Section 6.5 summarizes these rates of prenatal mortality at different periods during pregnancy in primate and non-primate species. Additionally, the species-specific genetic, maternal and environmental factors which contribute to prenatal loss are reviewed. Section 6.6 describes the genetic differentiation of the development of the reproductive tract and the sexual differentiation of both the reproductive tract and the brain. Sexual dimorphism of brain function as well as critical time periods for hormone action in sexual differentiation and morphology are also covered, particularly in relation to understanding abnormal development, which is dealt with in section 6.7. We emphasize in this section the major malformations of the reproductive system of animals and man and the ways in which experimental teratology can advance our knowledge of embryonic and fetal abnormalities. The teratogenic role of sex steroids and environmental agents receives special attention because of the current and future concerns regarding these compounds in abnormal development.

Finally, in section 6.8 we note briefly some of the more strategic and applied dimensions in this field of research concerning its application towards transfer and storage of gametes, the regulation of fertility either to stimulate or to inhibit reproduction in the human and the potential for the accelerated breeding and conservation of endangered species.

6.2 EMBRYO–MATERNAL INTERACTIONS

6.2.1 GENERAL

In all primates, ovulation occurs after a distinct follicular phase that does not overlap, in terms of oocyte maturation, with the previous luteal phase. This contrasts with the sheep and other non-primate species in which follicular maturation is in a late stage of preparation by the end of the luteal phase (Baird *et al.*, 1975). Ovulation takes place 12–36 h after the mid-cycle surge of LH and the oocyte continues to mature as it enters the Fallopian tube, with fertilization occurring 12–24 h later.

After fertilization, the embryo proceeds through cleavage stages that vary in timing according to species (McLaren, 1985; Chapter 2 of this volume). The timing of developmental stages in primates is shown in Table 6.1.

The embryo reaches the uterus on days 3–4

Table 6.1 The principal stages of implantation and their approximate timing (days) after ovulation in the human, baboon, rhesus monkey and marmoset monkey

Stage	*Human*	*Baboon*	*Rhesus*	*Marmoset*
Embryo enters uterus	3–4	4	4	4
Zona pellucida shed	5–6	7–8	7–8	9–10
Attachment	6–7?	8–10	8–10	11–12
Trophoblast differentiates (previous stages)	7–12	10–25	10–30	12–40
Tertiary villi	16–17	23–25	23–25	40+
Decidual reaction	12+	Local oedema (11–14)	Plaque (11–14)	Epithelial 20+ Stromal 30+
Implantation type	Interstitial	Superficial	Superficial	Superficial

of pregnancy when it is at the morula stage. Development to blastocyst, hatching from the blastocyst and attachment to the maternal endometrium take place between days 7 and 12 after ovulation depending on the species. Figure 6.1 shows the principal factors involved in the establishment of a dialogue between embryo, endometrium and corpus luteum at the start of pregnancy in primates.

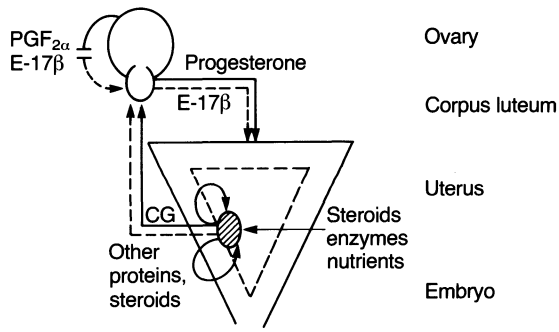

Figure 6.1 The embryo–maternal dialogue during implantation in primates. Pregnancy depends on progesterone from the corpus luteum and oestradiol may have a facilitatory role in stimulating endometrial support of the conceptus. The effects of ovarian prostaglandins or oestradiol-17β in terminating the lifespan of the corpus luteum need clarification. The embryo secretes CG to support the corpus luteum, and other proteins and steroids whose precise functions are not determined. Local actions of CG on embryo and trophoblast differentiation at implantation are conjectural.

It is at the time of attachment that CG is first secreted in measurable quantities, but this hormone, which is the first clear endocrine signal of the presence of an embryo, cannot be measured in the peripheral circulation for a further 2–3 days (as summarized in Table 6.2).

This vital attachment of embryonic to maternal tissue (see Figure 6.1), followed by rapid differentiation of trophoblast and invasion of the endometrial epithelium, must be completed efficiently in order to allow the establishment of vascular connections that ensure nutrient exchange to the embryo. Just as essential for the survival of the embryo is the rapid establishment of contacts whereby CG is conveyed to the corpus luteum to prevent its regression at the end of the cycle.

Table 6.2 The first detection (approximate days after ovulation) of CG in peripheral plasma for five primate species

Species	*Embryo attachment*	*CG first detected*
Human	7–9?	10
Chimpanzee	7–9	11
Baboon	8–10	12
Rhesus	8–10	12
Marmoset	11–12	14

Table 6.3 Factors affecting reproductive capacity in selected primate species (data for non-human primates are from captive colonies)

Species	*Ovarian cycle (days)*	*Gestation (days)*	*Average no. of young born/year*	*Sexual maturity (years)*	*Generation interval (years)*	*Seasonal breeding*[a]
Common marmoset (*Callithrix jacchus*)	28	144	5	1·5	2	No
Owl monkey (*Aotus trivirgatus*)	16	133?	1	1·5–2·0	2–3	No?
Squirrel monkey (*Saimiri sciureus*)	9	150	1	3	3–4	No?
Rhesus monkey (*Macaca mulatta*)	27	168	1	3–5	4–6	Yes
Baboon (*Papio cynocephalus*)	33	184	0·8	5–7	6–10	No?
Chimpanzee (*Pan troglodytes*)	37	235	<0·5	7–10	7–15	No
Man (*Homo sapiens*)	30	280	<0·5	15+	20+	No

[a]Of the species listed, the rhesus has a definite breeding season which may be extended but not abolished in captivity. Births will occur throughout the year in captive owl monkeys, squirrel monkeys and baboons, but there are probably seasonal changes in fecundity.

Within the general pattern summarized previously, there are considerable species differences among primates. These include the timing of attachment, the rates and degree of trophoblast invasion and the attainment of fetoplacental autonomy when the embryo and the extraembryonic membranes no longer require the presence of the corpus luteum for pregnancy to continue.

6.2.2 SPECIES DIFFERENCES

(a) Reproductive biology

Table 6.3 shows some factors affecting the basic reproductive biology and fecundity of selected primate species.

From these data we can see that studies of embryonic development are virtually impossible in some species, and not easy in any. The great apes are all threatened or endangered species and there are very few centres where it is possible to study their embryology in any detail. In contrast, there are now a number of centres in developed and less developed countries where the capacity exists for studies of simian species, both New and Old World.

(b) Morphology

In the human, the chimpanzee and presumably in the other ape species, the embryo and trophoblast sink well under the uterine epithelium, becoming surrounded quickly by endometrial tissue and maternal vasculature. In the simian species studied to date, the degree of invasion of trophoblast is less marked, the early endometrial hyperplasia is less evident and the embryo does not sink under the uterine epithelium although this layer is penetrated over a wide area by trophoblast (J.P. Hill, 1932; Wislocki and Streeter, 1938; Hertig *et al.*, 1959; J.D. Boyd and Hamilton, 1970; Hendrickx, 1971; O'Rahilly, 1973; Luckett, 1978). Figure 6.2 gives a diagrammatic comparison of the differences seen.

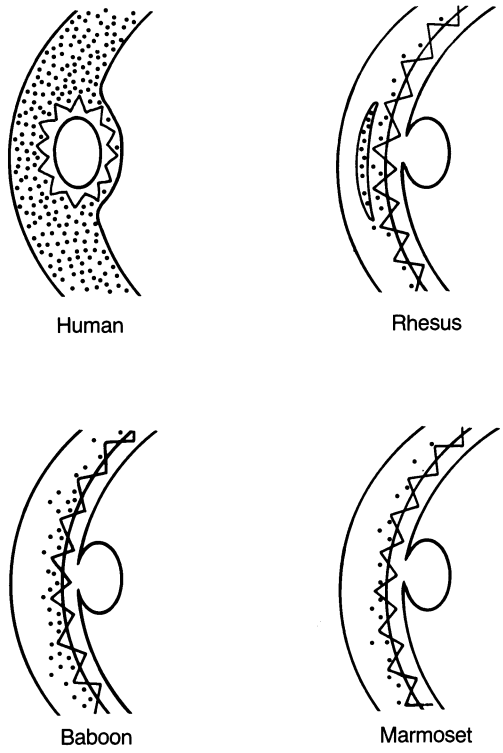

Figure 6.2 Implantation in primates, which requires further definition from ultrastructural investigations. The human trophoblast sinks under the endometrial epithelium and there is a massive endometrial reaction. In the monkeys studied to date, implantation is superficial, although rapid contact is made with the maternal vasculature. The degree of endometrial response varies in intensity and timing according to the species. Endometrial response; trophoblast–maternal interface; e.p. = epithelial plaque.

There have been more detailed accounts of the process of embryo implantation in the baboon and rhesus monkey (Hendrickx and Enders, 1980; Enders *et al.*, 1983) and in the marmoset monkey (H.D.M. Moore *et al.*, 1985; Smith *et al.*, 1987). From these studies it is clear that the stages of early embryonic development after embryo attachment occur relatively slowly in the marmoset when compared with the human and other primates. This may be due to the rather complex formation of early embryonic membranes in the marmoset, related to the chimaerism seen in this species (Figure 6.3).

The essential differences between the morphology of attachment and implantation in the hominoid and simian primates are perhaps academic in that after the first few days of implantation the degrees of embryo–maternal tissue apposition and exchange are similar whether the form of implantation is interstitial or superficial.

(c) The factors affecting the timing of embryonic development and survival

A comparison in selected species of the day of embryo attachment and the first day when CG is measurable in the peripheral circulation is presented in Table 6.2. Further comparisons on the rates of development of primate embryos are shown in section 6.4.

A major obstacle in obtaining data of this nature is that, until recently, it was almost impossible to provide a precise timing of the stage of development related to the time of ovulation. It is only since the wider use of radioimmunoassays in the 1970s and their application in monitoring the endocrine events of early pregnancy that a relatively non-invasive way of estimating the chronology of peri-implantation events has become feasible. This trend is continuing now with application of high-precision ultrasonography, which makes it possible to determine the day of embryo attachment through study of the size of the uterine lumen. Comparisons of embryo events based on the classic work of Hertig *et al.* (1956) and others depended on a very few samples from the human. Timing was related to the start of the previous menstrual period, so it is remarkable how much has been learned from this material. Definition of embryonic timing for the baboon can be achieved with greater precision because of the cyclical changes in sex skin exhibited in this species (Hendrickx, 1971). In other simians, including the rhesus monkey (Atkinson *et al.*, 1975), cynomologus

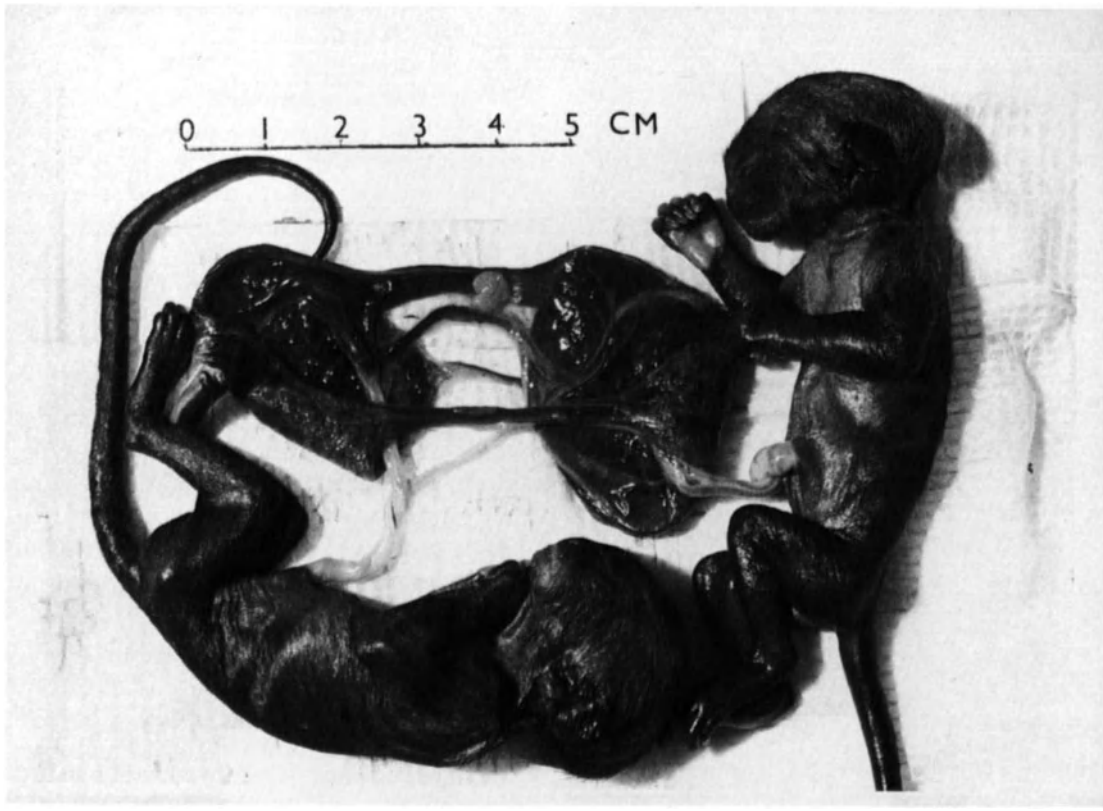

Figure 6.3 The anastomoses and vascular connections between triplet fetuses and placental discs in the common marmoset.

monkey (Dukelow, 1983) and marmoset (Hearn, 1983; Harlow *et al.*, 1984), daily monitoring of endocrine changes is necessary.

All of the above primate species have attributes and advantages depending on the questions being investigated. None of them are perfect models with homology to the human, but all share analogous attributes with the human in a general primate pattern. With regard to timing of the start of pregnancy, the marmoset has a particular advantage in that the end of the luteal phase can be synchronized by the administration of 0.5 μg of exogenous prostaglandin $F_{2\alpha}$ (Summers *et al.*, 1985). This permits collection and examination of embryos at precise stages of pregnancy as well as embryo transfer to synchronized recipients. This sensitivity of the corpus luteum and its possible evolutionary implications are noted further in section 6.3. Aspects of the evolution of the corpus luteum are discussed in Hearn *et al.* (1977).

The stage of embryonic development and the location of the embryo in the reproductive tract during the first 12 days of pregnancy in the marmoset monkey are shown in Table 6.4. As noted previously, the day of attachment of the embryo to the endometrial epithelium varies considerably between primate species (Table 6.1). Because of the obstacles in obtaining precise timing, it is likely that we may have to review our previous estimates of timing of human embryo attachment and implantation. Data from Lenton and Woodward (1988) can be interpreted to suggest that embryo attachment occurs on

Table 6.4 Embryo collection success rate and stages of embryonic development *in vivo* in the common marmoset

Day of pregnancy	*No. of laparot-omies*	*No. of CL*	*No. of embryos*[a]	*UF/deg. embryos*	*Stages of embryonic development*												
					Number of blastomeres												
					4	*5*	*6*	*7*	*8*	*10*	*10–16*	*16–20*	*M*	*EB*	*B*	*ExB*	*HB*
4	49	113	76 (67·3%)[b]	20	28	7	5	6	7	3							
5	2	5	4	0							3	1					
6	7	18	13	0							5	8					
7	29	69	52 (75·4%)[b]	8									35	9			
8	21	44	36 (81·8%)[b]	4									14	8	10		
9	32	79	60 (75·9%)[b]	4										5	18	33	
10	8	19	12	3												5	4
11	1	3	0														
Total	149	350	253 (72·3%)[b]	39													

[a]Includes unfertilized oocytes and degenerated embryos (UF/deg.).
[b]Number of embryos expressed as a percentage of number of corpora lutea.
M=morula; EB=early blastocyst; B=blastocyst; ExB=expanded blastocyst; HB=hatched blastocyst.

days 8–9, rather than the days 6–7 suggested by earlier investigations based on imprecise immunological assays that may not have distinguished fully between CG and LH (Saxena *et al.*, 1974). On the other hand, the highly invasive mode of implantation in the human may enable a quicker establishment of vascular connections, proving the earlier estimates correct. If the recent data are confirmed, it would unify the timing of implantation of Old World species, with attachment occurring on days 8–9 in the human as well as in the rhesus monkey and baboon.

The timing of implantation in New World species has been worked out in detail only in the marmoset, occurring on days 12–13 after ovulation (H.D.M. Moore *et al.*, 1985). It may be significant that the luteal phase of the marmoset at 19 days (Hearn, 1983; Harlow *et al.*, 1984) is also longer than the 14–15 days seen in most Old World species. On a superficial assessment, the day of embryo attachment in New World species may be quite flexible since the owl monkey (*Aotus trivirgatus*) has an ovarian cycle of 16 days with a luteal phase of approximately 10 days (Dixson, 1983); the cebus monkey (*Cebus apella*) 21 and 12 days respectively (Nagle and Denari, 1983); the tamarins (for example, *Saguinus oedipus*) 16 and 10 days; and the squirrel monkey is reported to have a complete cycle within 8–10 days (Dukelow, 1983).

Another factor that affects the timing of early embryonic development in the marmoset monkey is the absence of post-partum lactational amenorrhoea. The marmoset can become pregnant within 10 days of a previous birth. While this greatly increases the fecundity and the productivity of the marmoset, it results in an overlap between the first 80 days of pregnancy and the suckling of twin infants (Hearn, 1983, 1984). This double physiological load may explain in part why the first 80 days of marmoset embryonic development are relatively slow compared with the rates of development in the human, rhesus monkey and baboon (Chambers and Hearn, 1985). Figure 6.4 and Table 6.5 show the comparison between these four species. In all other primate species that have been studied in detail to date, there is a lactational amenorrhoea during which ovarian cyclicity is suspended.

A further limiting factor to fecundity

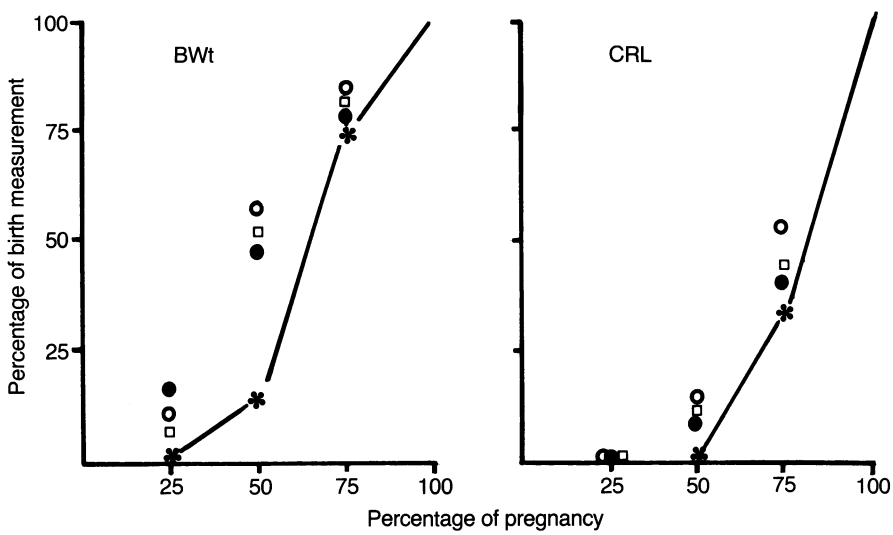

Figure 6.4 The percentage of the birth body weight (BWt) and the birth crown–rump length (CRL) achieved 25, 50 and 75% through gestation. Man (□), rhesus (●), baboon (○) and marmoset (*).

that will have shaped the evolution of reproductive strategies is that of seasonal breeding. Those primates, for example the marmoset and cynomologus monkeys, that show no seasonal periodicity in their reproduction are not constrained in their gestation length and lactation periods. Those that are tied to strict seasonal breeding, such as the rhesus monkey, need to complete a full gestation and lactation over 1 year if they are to breed when the next season starts. If, through adverse conditions of climate or diet, pregnancy starts too late in one season or lactation is prolonged beyond the start of the next, there is a negative effect on productivity. The infant–maternal interactions at weaning and the way in which these are related to reproductive productivity and the relative dominance of females in groups of baboons were studied by Nicolson (1981) and are reviewed by Lee (1987).

Embryonic loss is a significant factor affecting fertility and productivity in primates. Studies from non-primate species show that this is a phenomenon in many domesticated animals (Wilmut *et al.*, 1986). Estimates in the human vary from 20% to 50% loss of embryos as a normal, natural phenomenon (R.V. Short, 1979). Recent studies of the phenomenon related to the more controlled albeit less natural procedures of *in vitro* fertilization (IVF) and embryo transfer show that premenstrual pregnancy loss is significant in the human (Lenton *et al.*, 1988). The potential causes of embryonic loss are explored in more detail in section 6.4. It is difficult to obtain precise figures for normal embryonic loss during pre-implantation stages in the human, but it is likely that an estimate of 40% would not be far off reality. An understanding of the causes of this loss and the degree to which it is caused by genetic abnormality or by accidental failure of synchrony in the embryo–maternal dialogue could provide new initiatives in the treatment of human infertility, the regulation of human fecundity and the accelerated breeding of threatened and endangered species.

The current debate concerning the ethics of research on early embryos has a significant bearing on all of the above questions. The success of IVF procedures in some laboratories is now approaching the efficiency of natural embryonic survival. Further improvement for the human and extension of this knowledge to endangered primates depends on a greatly improved understanding of early embryonic development. At present, most of these questions can only be approached through experimental research over the peri-implantation period. The status of the embryo and the philosophical validity of the concept of a pre-embryo to describe the embryo's condition up to the 14th day of pregnancy needs further definition in drawing the boundaries of permitted research on the human embryo (McLaren, 1984, 1988; N. Ford, 1988).

Table 6.5 A comparison of the early embryonic development of the human, rhesus, baboon and marmoset showing the relative days of gestation in non-human species related to the same embryonic stage in the human

Species	*Implantation*	*Primitive streak*	*29–31-day human embryo*	*50-day human embryo*
Human	6–7	18	29–31	50
Rhesus	8–9	17	24	45
Baboon	8–9	17	27	46
Marmoset	12	30	60	80

6.2.3 EMBRYONIC SIGNALS AT IMPLANTATION

The first well-defined secretion of the early embryo that is known to be essential for its survival is chorionic gonadotrophin (CG). This glycoprotein, gonadotrophin hormone, is composed of α- and β-subunits, as are the other gonadotrophins and TSH (see this volume, Chapter 5). The biological activity of the gonadotrophins resides in their β-subunits, and a comparison of the CG and LH molecules shows the two to be virtually identical except that CG has an additional C-terminal chain of 32 amino acids (Canfield *et al.*, 1971; Morgan *et al.*, 1973).

The corpus luteum is supported by LH during the luteal phase and declines if monkeys are treated with antagonist to LHRH (Fraser *et al.*, 1985, 1986; Hodges *et al.*, 1988). There is a brief period in early pregnancy when the embryo must attach, activate the secretion of CG and maintain the production by the corpus luteum of essential progesterone and facilitatory oestrogen if pregnancy is to continue. The primate system therefore appears relatively simple in that a viable embryo controls its own survival from a very early stage by secreting sufficient CG to replace the luteotrophic effects of LH. It is likely that the secretion of CG also causes a reduction in LH secretion from the pituitary (Lenton and Woodward, 1988).

In the 20 years since the subunit structure of CG was established (Canfield *et al.*, 1971; Pierce *et al.*, 1971), thereby explaining the lack of specificity between antisera raised against the entire gonadotrophin hormones, there have been major advances in the precision of diagnostic applications related to pregnancy and to CG-secreting cancers. The availability of the amino acid and therefore the DNA sequence for human CG has enabled far more detailed study of its function. An understanding of the physiological significance of CG in pregnancy has been possible for many years at a peripheral, systemic level, but the studies at a cellular and molecular level that elucidate the local role of CG are only beginning to appear. This is not surprising since the availability of primate embryonic material is very limited; the capacity to study dynamic questions *in vivo* is hindered by the poor accessibility of the implantation site and of the corpus luteum; and *in vitro* culture of primate embryos through the whole peri-implantation process, together with the measurement of CG secretion, has only been achieved recently for the human (Fischel *et al.*, 1984), the baboon (Pope *et al.*, 1982) and the marmoset (Hearn, 1983; Hearn *et al.*, 1988c). The results of these and other studies are now posing leading workers to question whether CG's role is restricted to the traditional one of support of the corpus luteum until the end of the luteoplacental shift (Csapo *et al.*, 1972; Hearn, 1979; Ross, 1979). There is now a growing body of circumstantial evidence to suggest that CG may have a local role at the implantation site (Hearn, 1986), within the embryo itself (Hearn *et al.*, 1987, 1988a,b) or on early sexual differentiation of the primate gonad (Jost, 1970; Muller *et al.*, 1978a).

The profile of the secretion of CG through human pregnancy (Figure 6.5) might suggest that there are other functions for this hormone, since its secretion is still at relatively low levels by week 7 of pregnancy when its function of supporting the CL can be taken over completely through the secretion of progesterone by the placenta (Csapo *et al.*, 1972).

The specificity of CG to primate pregnancy has been challenged over many years. It is clear that this hormone is secreted by various cancerous tissues and that it can be used as in diagnostic and monitoring techniques in these cases. However, it now seems clear that CG is restricted to primates, since this order is the only one with the multiple gene system for control of CG, with one gene for the α-subunit and six or seven genes for the β-subunit (Talmadge *et al.*, 1984a,b; Policastro *et al.*, 1986). Consequently, the many claims

that have been made for CG secretion by embryos from species as diverse as the rat (Haour *et al.*, 1976) to the hyrax (Gombe *et al.*, 1980) may require re-examination. The other species in which a CG is expressed, the equids, do not actually express a specific placental gene since the protein synthesized by the cells of the chorionic girdle is identical to amino acid sequence in both its α- (H.J. Stewart *et al.*, 1987) and β-subunits (Bousfield *et al.*, 1987; Sugino *et al.*, 1987).

Chorionic gonadotrophin-β may be used as a trophoblast marker. Its expression in the embryo is thought to be limited to the syncytiotrophoblast cells of the chorionic villi, but the embryonic transcriptional activity of the genes may be observed much earlier since there is expression of hCG-β RNA at the six to eight-cell stage, before differentiation between trophectoderm and inner cell mass. This RNA is thought to be the product of *de novo* transcription from the embryonic genome since transcripts are observed only in embryos at least 2 days after fertilization (Bonduelle *et al.*, 1988).

In our own studies, we have concentrated on examining the role of CG *in vivo* and *in vitro* in the marmoset monkey. This has required the development and detailed validation of gonadotrophin and steroid assays in order to measure hormones in plasma, urine, tissues, cells and culture fluids. It was necessary to develop protocols for the synchronization of early pregnancy events using $PGF_{2\alpha}$ and hCG to stimulate ovulation; surgical and ultrasound procedures for the monitoring of follicular development and implantation, recovery, storage and transfer of embryos (Hearn, 1980, 1983; Summers *et al.*, 1985, 1988); morphological confirmation of the stages of implantation at light and electron microscopic levels (H.D.M. Moore *et al.*, 1985; Smith *et al.*, 1987); *in vitro* embryo manipulation and culture systems (Hearn *et al.*, 1988a; Summers *et al.*, 1988); and systems for microcannulation of the corpus luteum *in vivo* (Hearn and Webley, 1987; Webley and Hearn, 1987) as well as *in vitro* culture of marmoset and human granulosa and luteal cells (Webley *et al.*, 1988, 1989a).

Once the above systems had been developed and tested, we could ask questions about embryo–maternal interactions during the peri-implantation period at the levels of the whole animal, the cell recognition systems and now at the molecular level. These investigations could determine some of the factors regulating the secretion of CG, and the functions of this hormone, through its immunological neutralization. A summary of studies *in vivo* and *in vitro* is provided below, with reference to the work of other investigators.

(a) *In vivo*

Figure 6.5 shows the measurement of CG in the peripheral plasma of the marmoset during early pregnancy. In keeping with the results shown in Table 6.2, there is a period of 2–3 days after embryo attachment before a significant rise in peripheral levels can be detected, and it is not possible to deduce exactly, *in vivo*, when CG starts to be secreted by the embryo. Active or passive immunization of marmosets during early pregnancy against CG will inhibit implantation and terminate the pregnancy, suggesting that this hormone is necessary for successful implantation and survival of the embryo (Hearn, 1975, 1976, 1978, 1979; Hearn *et al.*, 1976).

Similar results were reported for active immunization in the baboon (V.C. Stevens, 1976) and the rhesus monkey (Talwar *et al.*, 1976). These results formed the basis for a research programme carried out by WHO and the Population Council to explore the feasibility of a vaccine against CG β-subunit or synthetic fragments of the C-terminal polypeptide chain as a long-term contragestational agent in the human. The research is currently being extended to phase II clinical trials in India and Australia.

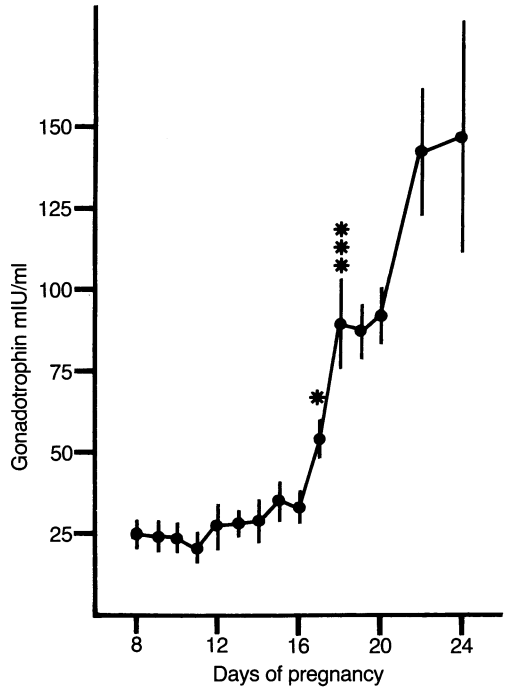

Figure 6.5 Concentrations (mean ± SEM) of CG in peripheral plasma of marmoset monkeys (n=10) during the first 24 days of pregnancy (after ovulation).

(b) *In vitro*

Figure 6.6 shows the production of CG by marmoset embryos cultured *in vitro* on a layer of marmoset fibroblasts (Hearn, 1983; Hearn *et al.*, 1988a).

The results indicate that CG secretion is not switched on or detectable, external to the embryo, until the time when the embryo attaches to the feeder layer and starts to differentiate into embryo proper, yolk sac and trophoblast. This is analogous to the time of attachment at the start of implantation *in vivo* (Figure 6.7). This conclusion was tested more rigorously by separation of inner cell mass and trophoblast in bisected embryos, suggesting that the inner cell mass is necessary for the efficient initiation of CG secretion (Hearn *et al.*, 1988b,c). Once again CG secretion was not detectable in culture fluids until after embryonic attachment. The intraembryonic role of CG requires further examination, as does its potential role in early embryonic differentiation and establishment of the implantation site (Hearn *et al.*, 1987). Summers *et al.* (1987) were able to establish a trophoblast cell line derived from the inner cell mass, subcultures of which have continued to produce CG for over 5 years. The prospects for working with primate embryonic cell lines and stem cells using this system are of great interest.

In a preliminary experiment with limited material, embryos cultured from the blastocyst through the *in vitro* 'implantation' system noted above were prevented from successful attachment if culture fluids contained marmoset anti-hCG-β antiserum at levels calculated to be similar to those that were effective in blocking implantation *in vivo* (Hearn *et al.*, 1988b). Although tentative, the results suggested that CG may be necessary for the process of attachment itself. We have yet to examine the expression of CG on the embryo surface immediately before attachment, since in spite of considerable effort we have yet to achieve a fully purified marmoset CG-β. It would be no surprise to find such expression since transcriptional activity of the encoding gene is thought to be present in the human embryo by the six to eight-cell stage (Bonduelle *et al.*, 1988). Fishel *et al.* (1984) and Lachtan-Hay and Lopata (1988) imply that the human pre-implantation embryo is capable of secreting CG into culture fluid, but it is not clear whether some contamination with LH might have been possible.

Although the studies we report in the marmoset do not show clear secretion of CG before attachment, recent studies in the rhesus monkey (Seshagiri and Hearn, 1993; Seshagiri *et al.*, 1993) and human (Hay and Lopata, 1990; Dokras *et al.*, 1991) have shown low levels of CG to be secreted before attachment, with an exponential increase commencing immediately after attachment. In addition, we have tested the hypothesis that

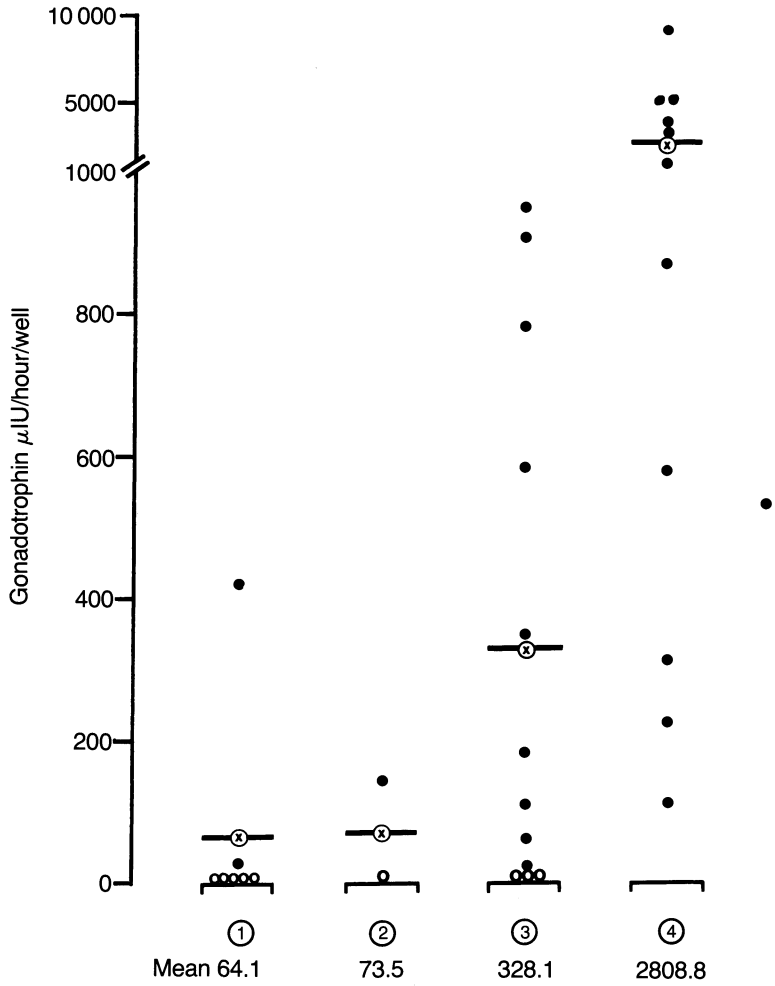

Figure 6.6 The secretion of CG by marmoset embryos cultured *in vitro*. Embryos were cultured for periods of 2–10 days. The sampling times are (1) blastocyst – hatched blastocyst; (2) hatched blastocyst–attachment; (3) attached blastocyst–48 h after attachment; and (4) 48 h after attachment–120 h after attachment.

CG in the preattachment embryo may be regulated by intraembryonic gonadotrophin-releasing hormone (GnRH) in a system analogous to the hypothalamo-pituitary regulation of gonadotrophins. Our results show for the first time that GnRH is expressed and secreted by the pre-implantation primate embryo, in a close association with the timing and secretion of CG. We are currently testing the causal linkage between these two hormones (Seshagiri and Hearn, 1993, unpublished). If confirmed, a GnRH–CG regulatory system in the primate embryo may permit new approaches to our understanding of embryo loss and to the regulation of fertility. As far as we know, it is only the primates that have adopted this mechanism into the embryo at this early stage of pregnancy, raising interesting questions about the evolution of the physiological mechanisms involved in the

maternal recognition of pregnancy and the support of the corpus luteum (Hearn *et al.*, 1991).

We conclude from the above studies and from others that CG is an essential requirement for implantation, that this hormone is necessary for the rescue of the corpus luteum and that further work is required to confirm a local role within the embryo, at the embryo–maternal interface and in embryonic gonadal differentiation. These questions have been intriguing developmental biologists and now it looks as though the cellular and molecular tools of sufficient precision to provide answers are becoming available.

6.2.4 EMBRYONIC SIGNALS BEFORE IMPLANTATION

The studies summarized so far prompt one to ask whether any embryonic signals are necessary before implantation. The beautiful simplicity of a system in which the embryo produces its own gonadotrophin and therefore takes command of the corpus luteum and perhaps of its own differentiation within days of the start of pregnancy leaves little room for improvement.

There are a number of candidates as pre-implantation signals in mammals. In the pig it was shown clearly that oestrogen-17β secreted by the blastocyst is necessary before implantation (Perry *et al.*, 1973) but a comparative study showed that this steroid was apparently not so important in a range of other species (Gadsby *et al.*, 1980). In the sheep and cow the conceptus secretes a protein prior to attachment, called ovine or bovine trophoblast protein 1, which prevents luteal regression and shows homology with α – interferons (Imakawa *et al.*, 1987, 1989; H.J. Stewart *et al.*, 1987; Charpigny *et al.*, 1988).

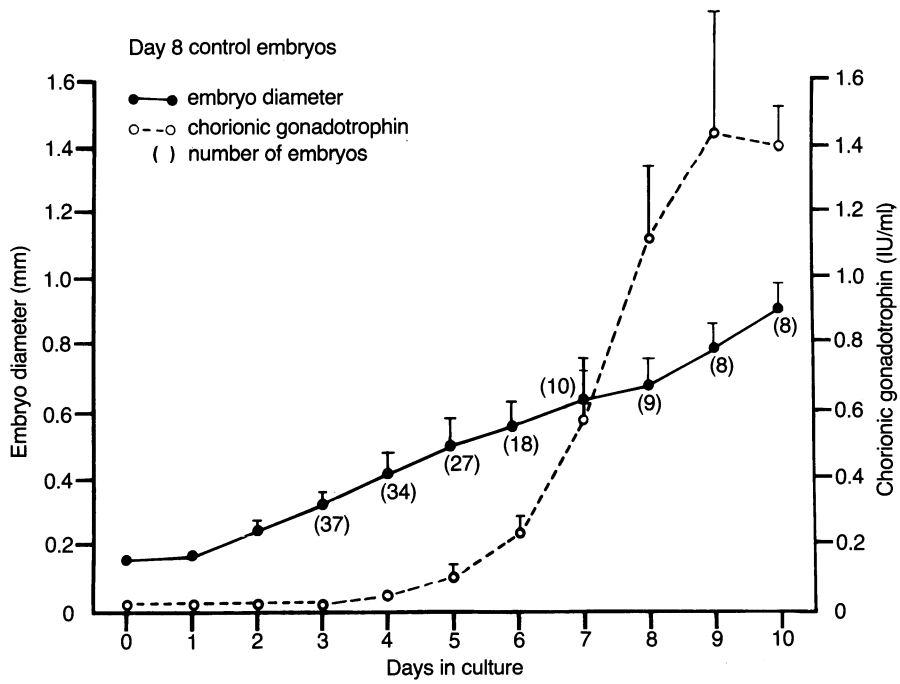

Figure 6.7 The growth in embryo diameter and the onset of secretion of CG in embryos recovered on day 8 after ovulation (blastocyst) and cultured for 10 days *in vitro*. Values are mean ± SEM for the number of embryos in parentheses.

Three further candidates, each claimed to be expressed soon after fertilization, include early pregnancy factor (EPF) for a range of mammalian species including mouse and man (Morton *et al.*, 1983), platelet-activating factor (PAF) as a phospholipid implicated in the establishment of pregnancy (O'Neill, 1985) and an embryo-derived histamine-releasing factor (Cocchiara *et al.*, 1987). No doubt there will be many more claims over the next few years for a range of growth factors, proteins or steroids, and it will be a challenge to tease out those that play an essential role, those that are facilitatory and those that have minor relevance. The same is likely to be true for secretions of the oviduct and uterus, for which there must be some significant factors, but their physiological actions in the embryo–maternal dialogue are too subtle for us to define with current technology. It is necessary to keep an open mind, while avoiding going on fishing trips without bait.

For example, when it was shown by Marston *et al.* (1977) that binucleate embryos could be transferred to the uterus of the rhesus monkey and implantation could proceed, some concluded that the oviduct was merely a tube and its secretions not essential. It was also concluded correctly that a precise synchronization of embryonic development during passage down the Fallopian tube in primates was not as necessary as in non-primates. The primate appears to be far more flexible than the rodent in this regard. This flexibility has been confirmed since by a great deal of the work in embryo transfer associated with human IVF (Edwards *et al.*, 1985) and also in primates such as the marmoset (Summers *et al.*, 1987). One result caused in part by Marston's finding was that there was a marked drop in funding for studies attempting to regulate fertility by interfering with the rate of passage of the embryo through the oviduct. This was at a time when Ortiz and Croxatto (1979) were obtaining some fascinating data about the early stages of embryonic development in the human and the timing of differentiation events. Their studies *in vivo* and those of Edwards, Trounson and others at the time, and the whole field of IVF subsequently, have given us a more considerable amount of knowledge in the human about pre-implantation stages of development than has been achieved for any other primate species. To that extent the human is now the model species for other primates.

The efficiency of embryo growth *in vitro* is relatively poor. In contrast to the successful media that have been developed for rodent embryo culture (Biggers, 1983; Balmaceda *et al.*, 1986; Bavister, 1987; Bavister and Boatman, 1989), culture systems for primate embryos give low embryonic survival to implantation if they are maintained for more than 2–3 days (reviewed by Bavister, 1987). Some findings indicate that co-culture of non-primate embryos with a monolayer of oviductal cells greatly increases the stability of the system and the viability of the embryo (Gandolfi and Moor, 1987; C. Polge, personal communication, 1989). These results suggest that the oviduct does have a role to play in facilitating embryonic development. The factors affecting formation and function of oviduct fluid are now becoming more apparent and may be found in a useful review by Leese (1988). This knowledge is vital if we are to understand the requirements for media for the culture of primate embryos. How these factors are involved in the embryo–maternal dialogue, as essential or subordinate, and whether they are a necessary stage in preparation of the uterus for implantation, remains to be seen.

The very obvious advantages that could be exploited both scientifically and commercially if any pre-implantation signal was defined have exerted considerable pressure to generate rapid results in this area of enquiry. A signal that could be utilized as a very early diagnosis of pregnancy, as a monitor of embryo health and viability or as the basis of an

inhibitory contragestational approach to fertility control, would be ideal in an area in which development of concepts has occasionally outstripped the realities of fully controlled experimental results.

The effects of bidirectional signals during pre-implantation life are now becoming apparent in several non-primate species (reviewed by Heap *et al.*, 1988). PAF and EPF are claimed to be present in addition in the human. O'Neill *et al.* (1985) showed that a transient thrombocytopenia could be induced in association with PAF, with chemical and physiological similarities to an acetylated choline ether lipid, the effects of which are evident within 6 h of fertilization and extend through pre-implantation development. This lipid shows many characteristics of a mediator and is ubiquitous in its production by cells, with receptors in many tissues, having a role as an intercellular messenger. PAF can exert a local effect and it does not normally enter the peripheral circulation. The studies reported by O'Neill (reviewed by O'Neill, 1987) are quite clear-cut, but as yet attempts to extend this system have proved ambiguous. Our own studies in the marmoset monkey, referred to in Hearn *et al.* (1988b,c), showed a reduction in the numbers of circulating platelets during the pre-implantation phase, but there was considerable intra- and inter-individual variation that was too high to allow us to use this system as a diagnostic or to inspire confidence in the feasibility or precision of a laborious methodology. The variations reported by three observers using the same methods with the same samples was not acceptable. We concluded that there was an association between thrombocytopenia and pre-implantation stages of pregnancy in the marmoset monkey but that the current methodology is too imprecise to allow any interpretation of cause and effect, at least in our hands. When there is a robust assay, we look forward to testing the assertion further (Hearn *et al.*, 1988b,c). A link between PAF and EPF was shown by Orozco *et al.* (1986), the former inducing expression of the latter within 1 h after injection. The potential of EPF and many hypotheses concerning its subunit structure and function have been explored enthusiastically in the literature over the past 15 years. It is fair to say that at the end of this period the prospects remain just as intriguing and just as much a mirage. The detection of EPF depends on a delicate *in vitro* test involving a small population of T lymphocytes in circulation to bind heterologous red cells in the presence of complement, this being inhibited by antibody (Morton *et al.*, 1980; Heap and McRae, 1988). Once again the rigorous testing of hypotheses must await the development of far more robust and precise assays.

The peri-implantation period is clearly a time when many genes are activated, but it is more difficult to assign physiological function to the gene products when they are expressed. Considerable amount of circumstantial evidence supporting a pre-implantation embryo–maternal dialogue will require confirmation at a molecular level as well as definition of the functions. In primates this is extremely difficult because of the minuscule amounts of embryonic material that are available. Advances in technology, including the PCR reaction, will make such studies feasible in the next few years.

6.2.5 MATERNAL SIGNALS DURING THE PERI-IMPLANTATION PERIOD

During its passage down the Fallopian tube and the establishment of vascular connections with the endometrium, the embryo requires nutrients that are provided by the oviduct and uterus. These nutrients and other factors are controlled through the secretion of progesterone and oestrogen by the corpus luteum. In primates, the dominant of these hormones is progesterone, and pregnancy will proceed if the corpus luteum is removed and adequate exogenous progesterone is provided in the rhesus monkey

and in women (Finn, 1985, and this volume, Chapter 3). It would be surprising if oestrogens did not facilitate the process of implantation in primates, but in rodents oestrogen is essential for implantation to occur (Finn, 1985).

Active and passive immunization against progesterone and oestrogen has been carried out during early pregnancy in a wide range of species, causing either partial or complete disruption (Hillier and Cameron, 1976). In recent times the approach has become far more precise, with Heap and his colleagues showing that passive immunization of mice with a monoclonal antibody against progesterone will block implantation and may inhibit embryo growth during the pre-implantation phase (Wright *et al.*, 1982; Ellis *et al.*, 1988). In addition, mouse monoclonal anti-progesterone IgG1 antibody can be seen, using rabbit polyclonal anti-idiotype in indirect immunofluorescence, on the surface of uterine luminal and glandular epithelia. This suggests that anti-progesterone antibody binds to an antigen that is associated with the uterine surface and that inhibits locally the uterine uptake of progesterone. Antisera to progesterone have provided similar results in rats and ferrets (Rider and Heap, 1986) but have yet to be extended to primates. A most useful summary of the maternal reactions affecting early embryogenesis and implantation in non-primate species, including reference to growth factors, angiogenesis and maternal cellular reactions, is provided in Heap *et al.* (1988). This topic is also dealt with extensively by Finn in this volume in Chapter 3.

There are a number of other putative embryonic or maternal signals found in various species that are thought to be involved in implantation (for reviews see Bazer *et al.*, 1986; Heap *et al.*, 1988; Hearn *et al.*, 1988b,c). These include 'purple protein' in the rabbit (Chen *et al.*, 1975) and proteins secreted by the endometrium during peri-implantation stages in the sheep (Findlay *et al.*, 1979). There are also the many pregnancy-associated proteins that are derived from trophoblast or maternal tissue and whose regulation and physiological functions are uncertain (reviewed in Chapman *et al.*, 1988). We do not propose to go into any further detail here.

It is likely that in the next few years we will see a multiplication of the factors with a potential role in implantation. This will be a consequence of the ever more precise molecular technology available, resulting in a long list of gene products that appear and disappear during this crucial period in embryonic differentiation. The prospects for advancing our understanding of the fundamental control processes of embryogenesis are most exciting. We must ensure, however, that we also develop adequate *in vivo* and *in vitro* test systems to make physiological sense of these potential signals or much time and resources will be wasted in chasing signals of minor importance.

6.3 REGULATION OF THE CORPUS LUTEUM

The corpus luteum is a highly vascularized structure which is formed in the ovary after release of the egg at ovulation. Its main function is in the secretion of progesterone, and also oestradiol in the primate, which act on the endometrium to provide a uterine environment which can receive and support the developing embryo. The lifespan of the corpus luteum is dependent on the presence of the embryo within the uterus. In the absence of fertilization, the corpus luteum undergoes regression and progesterone production decreases. The length of the luteal phase in the non-conception cycle varies between species. In the primate the mean cycle length ranges from 8.70 ± 3 days in the squirrel monkey (*Saimiri sciureus*) (R.C. Wolf *et al.*, 1977; Dukelow, 1983) to 30.1 ± 3.8 days in the marmoset monkey (*Callithrix jacchus*) (Chambers and Hearn, 1979; R.D. Harding *et al.*, 1982; Harlow *et al.*, 1983) both New World

primates, with cycle lengths for Old World primates of 27 ± 1 days in the rhesus monkey (*Macaca mulatta*) (Monroe *et al.*, 1970) and 30.3 ± 3.1 days for the baboon (*Papio anubis*) (C.V. Stevens *et al.*, 1970). However, following fertilization in a conception cycle the levels of progesterone do not fall either in women (Corker *et al.*, 1976) or monkeys (Knobil, 1973) and the corpus luteum is maintained. The extension of the lifespan of the corpus luteum is essential in early pregnancy for the continued production of progesterone and maintenance of the uterus. It is achieved in the human and non-human primate by the action of chorionic gonadotrophin secreted by the implanting embryo (Knobil, 1973; D.T. Baird, 1985).

6.3.1 CORPUS LUTEUM REGRESSION

The mechanisms of luteal regression are not completely understood in primate species. This is in contrast to the mechanisms adopted by a number of other species which have been well characterized. In the following paragraphs the mechanisms of luteolysis will be reviewed and compared with results obtained to date in the human and non-human primate.

(a) Prostaglandin $F_{2\alpha}$-induced luteolysis

In many species, including the sheep and cow, luteolysis is induced by the action of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) released from the uterus (McCracken *et al.*, 1970; Flint and Hillier, 1975). Hysterectomy in these species prolongs the life of the corpus luteum (CL), as does immunization against $PGF_{2\alpha}$ (Fairclough *et al.*, 1981). $PGF_{2\alpha}$, secreted by the uterus, reaches the ovary via a countercurrent system in the ovarian pedicle (McCracken *et al.*, 1971, 1981; Bazer and First, 1983) and by local transfer from uterine lymphatic vessels to the adjacent ovarian vein (Heap *et al.*, 1985). $PGF_{2\alpha}$ is most effective in causing luteolysis when released from the uterus in an episodic manner (Thorburn *et al.*, 1973). The release of $PGF_{2\alpha}$ appears to be under the control of circulating oxytocin, secreted by the ovary and interacting with receptors in the uterine endometrium (Wathes and Swann, 1982; Sheldrick and Flint, 1985, Flint and Sheldrick, 1986).

In the primate, $PGF_{2\alpha}$ secreted by the uterus does not appear to initiate luteolysis since hysterectomy has no effect on cycle length. This has been demonstrated both in the rhesus monkey (Neill *et al.*, 1969) and in women (Beling *et al.*, 1970). Although a countercurrent system between the ovary and uterus has been described in women (Bendz *et al.*, 1979; Einer-Jensen *et al.*, 1985), it does not appear to be of major importance at the time of luteal regression.

Intraovarian production of $PGF_{2\alpha}$ has been postulated as the source of $PGF_{2\alpha}$ for the induction of luteolysis in the primate (Rothchild, 1981). Evidence for this is provided by the ability of luteal tissue, from the rhesus monkey and women, to synthesize prostaglandins (Swanston *et al.*, 1977; Valenzuela *et al.*, 1983). A luteolytic action of $PGF_{2\alpha}$ on human ovarian cells *in vitro* has been described (McNatty *et al.*, 1975; Dennefors *et al.*, 1982), intraluteal administration of $PGF_{2\alpha}$ to the rhesus monkey results in luteolysis (Auletta *et al.*, 1984a) and perfusion of $PGF_{2\alpha}$ through the corpus luteum of the marmoset monkey *in vivo* was associated with a decrease in progesterone production (Hearn and Webley, 1987).

Systemically administered $PGF_{2\alpha}$ causes luteolysis in many species (Horton and Poyser, 1976) but there are variable reports of the effect of $PGF_{2\alpha}$ given systemically to the primate. A single intramuscular injection (0.5 μg) of a $PGF_{2\alpha}$ analogue (cloprostenol) to the marmoset monkey, a New World species, is luteolytic when given after day 8 of the luteal phase or during pregnancy (Summers *et al.*, 1985). In contrast, similar dose/weight injections of cloprostenol to the baboon, an Old World monkey, are not luteolytic at any

stage of the cycle or pregnancy (Eley *et al.*, 1987). $PGF_{2\alpha}$ is only transient in effect when administered systemically to women (Wentz and Jones, 1973).

A proposed mechanism through which $PGF_{2\alpha}$ can cause luteolysis is by blocking cAMP-dependent LH stimulation of progesterone secretion. This action of $PGF_{2\alpha}$ has been demonstrated after short-term tissue incubations and long-term culture of luteal cells in sheep (Fletcher and Niswender, 1982), cow (Pate and Condon, 1984) and rat (J.P. Thomas *et al.*, 1978). $PGF_{2\alpha}$ has also been shown to block the uptake of labelled hCG by luteal tissue *in vivo* in the rat (Behrman and Hichens, 1976). A similar luteolytic mechanism for $PGF_{2\alpha}$ has been demonstrated in the primate. In women, using both granulosa cells luteinized *in vitro* (McNatty *et al.*, 1975) and incubated luteal tissue pieces (Hamberger *et al.*, 1980), $PGF_{2\alpha}$ blocked LH/hCG-stimulated progesterone production. In the marmoset monkey perfusion of the corpus luteum *in vivo* with $PGF_{2\alpha}$ prevented the stimulatory action of hCG perfused subsequently (Hearn and Webley, 1987) and, after short-term incubation of tissue pieces, $PGF_{2\alpha}$ prevented stimulation of progesterone secretion by hCG (Webley *et al.*, 1989b).

Not all studies have demonstrated a luteolytic action for $PGF_{2\alpha}$. Indeed, $PGF_{2\alpha}$ stimulates progesterone production by incubated dispersed luteal cells from the cow (Alila *et al.*, 1988). A similar action has been demonstrated in the primate. $PGF_{2\alpha}$ stimulated progesterone secretion by mid-luteal phase cells from the rhesus monkey (Stouffer *et al.*, 1979), by incubated human luteal cells (Richardson and Masson, 1980) and by luteal cells taken in the mid-luteal phase and early pregnancy from the marmoset monkey (Webley *et al.*, 1989a). As $PGF_{2\alpha}$ can inhibit LH/hCG-stimulated progesterone production by incubated tissue pieces from the human (Hamberger *et al.*, 1980) and marmoset monkey (Webley *et al.*, 1989b), this suggests the necessity for tissue integrity for the luteolytic action of $PGF_{2\alpha}$ to be evident, which is lost after cell dispersion. Also, the contribution of different cell types in the CL may be of importance, as has been shown in the bovine CL; $PGF_{2\alpha}$ stimulated progesterone production by small luteal cells but inhibited LH-stimulated progesterone secretion by large luteal cells (Alila *et al.*, 1988). Cell contribution may vary with cycle stage as the early CL is resistant to $PGF_{2\alpha}$-induced luteolysis in the sheep (Hearnshaw *et al.*, 1973) and pig (Bazer *et al.*, 1982) and also in women (Dennefors *et al.*, 1982), the rhesus monkey (Stouffer *et al.*, 1979) and the marmoset monkey (Summers *et al.*, 1985). Alternatively, $PGF_{2\alpha}$ in the human may require neural innervation of the CL for its luteolytic action, this innervation not being sufficiently developed in the early luteal phase (Dennefors *et al.*, 1982).

(b) Other luteolytic mechanisms

While other luteolytic agents have been postulated in the primate, their role has not been fully established. One such candidate is oestradiol, which has a variable effect on luteal function in different species (see Richardson, 1986). Oestrogens are produced by the primate CL and their role as the endogenous luteolysins has been suggested (Knobil, 1973; Auletta and Flint, 1988). Luteolysis was induced in the rhesus monkey after systemic administration of oestradiol in the later half of the luteal phase (Schoonmaker *et al.*, 1982). This probably resulted from an action both at the pituitary, to increase negative feedback on LH release, and at the ovary, but oestradiol can induce luteolysis in the rhesus monkey when administered directly into the corpus luteum without an associated decrease in LH concentrations (Auletta *et al.*, 1985). Oestradiol has also been shown to decrease basal and LH/hCG-stimulated progesterone production *in vitro* by luteal cells and tissue from the human (Richardson and Masson, 1981; Laherty *et al.*, 1985; Hahlin *et*

al., 1986) and rhesus monkey (Stouffer *et al.*, 1977). The effect was evident at high doses of oestradiol and, although these doses may correspond to the levels found within the CL (Swanston *et al.*, 1977), an increase in the luteal concentration of oestradiol was not recorded at the time of luteolysis. One suggestion is that oestradiol may cause luteolysis by stimulating intraluteal production of $PGF_{2\alpha}$ (Auletta *et al.*, 1978), although in human luteal cells indomethacin, an inhibitor of PG, did not inhibit the action of oestradiol (Thibier *et al.*, 1980). Another suggestion is that oestradiol may act by inhibiting the action of 3β hydroxysteroid dehydrogenase, the enzyme which converts pregnenolone to progesterone (Williams *et al.*, 1979). However, a clear role for oestradiol in the control of luteolysis in the primate remains uncertain, particularly since studies have demonstrated that administration of anti-oestrogens fails to prevent luteolysis (Westfahl and Kling, 1982; Westfahl and Resko, 1983) and additionally oestradiol receptors have not been detected in any cells in the macaque CL throughout the luteal phase (Stouffer *et al.*, 1989).

Another candidate for a luteolytic agent in the primate is oxytocin. However, the presence of and a role for oxytocin in the human CL has been disputed, as recently discussed by Auletta *et al.* (1988). While there are reports of the presence of oxytocin in the human (Wathes *et al.*, 1982; Dawood and Khan-Dawood, 1986), cynomologus monkey (Khan-Dawood *et al.*, 1984) and baboon (Khan-Dawood, 1986) CL, others have not been able to demonstrate the presence of oxytocin in either the human (Richardson, 1986), rhesus monkey or marmoset monkey (Auletta *et al.*, 1988). Also disputed is the action of oxytocin. Tan *et al.* (1982) showed that low doses of oxytocin (4 μg/ml) stimulated progesterone production by dispersed human luteal cells, whereas high doses (40 μg/ml) inhibited both basal and hCG-stimulated progesterone production, an effect confirmed by Bennegard *et al.* (1987) with intact human luteal tissue. Oxytocin is also luteolytic when infused directly into the corpus luteum of the rhesus monkey (Auletta *et al.*, 1984b). In contrast, Richardson and Masson (1985) did not observe any change in basal or hCG-stimulated progesterone production when dispersed human luteal cells were incubated with oxytocin. Wilks (1983) also failed to see any major effect of oxytocin after *in vivo* administration in the rhesus monkey.

GnRH or its analogues have been shown to directly inhibit gonadotrophin-stimulated progesterone production by rat luteal cells (Clayton *et al.*, 1979; Harwood *et al.*, 1980). Consistent with the response was the demonstration of specific binding sites (Clayton *et al.*, 1979; see Richardson, 1986). Specific binding sites for GnRH have been demonstrated also in the primate CL (Bramley *et al.*, 1985), but the effect of GnRH on steroid production by luteal tissue in the primate is unclear. In one study progesterone production was inhibited *in vitro* by a long-acting LHRH analogue (Tureck *et al.*, 1982), whereas in another neither GnRH nor its analogue appeared to influence steroidogenic function of human granulosa cells (Casper *et al.*, 1982). Since hypothalamic GnRH is unlikely to reach the ovary in sufficient quantities, a direct action for GnRH at the primate CL awaits identification of an LHRH-like peptide in the primate ovary.

Another mechanism whereby luteolysis could be induced in the primate would be to remove luteotrophic support. It has now been clearly demonstrated, using the effects of LHRH antagonists during the luteal phase, that the primate CL depends on support provided by pituitary LH (Fraser *et al.*, 1985, 1986; Hodges *et al.*, 1988). In both women and the rhesus monkey, studies have described a decrease in the pulse frequency and amplitude of LH at the end of the luteal phase, associated with reduced pulses of progesterone (Ellinwood *et al.*, 1984; Filicori *et*

al., 1984). However, it does not appear that a decrease in LH is the cause of luteolysis since induced reduction of LH pulses in rhesus monkeys, which have hypothalamic–pituitary lesions, had no effect on the length or function of the luteal phase. The possibility of a decrease in the binding of LH at the CL inducing luteolysis also appears unlikely as a decrease in LH receptors does not necessarily precede or associate with the decrease in progesterone at luteolysis (Cameron and Stouffer, 1982). The pattern of LH/hCG receptors follows the pattern of progesterone in the human luteal phase, reaching a peak in the mid-luteal phase and falling at the end of the luteal phase (Yeko *et al.*, 1989).

To conclude on the data obtained so far on the control of luteolysis in the primate, it is probably appropriate to conclude that the mechanism is still unclear. The intraluteal production of $PGF_{2\alpha}$ causing luteolysis is a likely candidate, however there are several discrepancies, including the absence of an increase in intraluteal concentrations of $PGF_{2\alpha}$ before the onset of luteal regression (D.T. Baird, 1985), the absence of an effect of indomethacin on the lifespan of the corpus luteum (Manaugh and Novy, 1976) and the absence of an effect of *in vivo*, administered $PGF_{2\alpha}$ in some primates (Eley *et al.*, 1987). In addition, a PG synthesis inhibitor, sodium meclofenamate, infused into the CL of rhesus monkeys caused premature luteolysis rather than extending the life of the CL, indicating luteotrophic rather than luteolytic actions for PGs (Sargent *et al.*, 1988). A paper on human luteal function (Fisch *et al.*, 1989) considered it unnecessary to evoke an active luteolytic mechanism. Instead it suggested that luteolysis is preprogrammed at the time of ovulation and is only reversed by hCG secreted by the implanting embryo in pregnancy. As the CL becomes increasingly dependent on LH support in the late luteal phase (Fillicori *et al.*, 1984) it is difficult to understand how the CL would retain its ability to respond to hCG but not LH when the receptors are similar. As previously stated, a decrease in pulsatile LH does not precede luteolysis. We would suggest from our studies on the human and marmoset monkey that there is an intraluteal luteolytic agent, probably $PGF_{2\alpha}$, to which the CL becomes increasingly susceptible as the luteal phase progresses. Recent evidence in the marmoset monkey (Michael and Webley, 1993) and rhesus monkey (Houmard *et al.*, 1992) suggests that the changing response of second-messenger systems to $PGF_{2\alpha}$ may determine the timing of luteolysis. The luteolytic agent acts to inhibit LH support, which is increasingly necessary for the function of the CL. It is not able to inhibit the luteotrophic action of hCG because of the larger size of the hCG molecule and the extent of LH/hCG receptor occupancy, which is >90% in early pregnancy as compared with 10–30% in the late luteal phase (Bramley *et al.*, 1987).

6.3.2 CORPUS LUTEUM 'RESCUE' IN EARLY PREGNANCY

In early pregnancy a message is secreted by the embryo which prevents luteolysis and enables the life of the CL to be extended for the continued production of progesterone. A variety of messages produced by the embryos of different species have been identified; these include proteins, steroids and prostaglandins. The mechanisms by which these messages prolong the life of the CL varies between species and will be discussed in the following paragraphs in relation to the information available in the primate.

(a) Luteotrophic messages

As described previously, the first clearly defined message to be secreted by the primate embryo is CG. CG has a direct luteotrophic action, stimulating the production of increasing amounts of progesterone from the CL, prolonging its life beyond that of the luteal

phase and transforming it into the CL of pregnancy (Knobil, 1973). Administration of exogenous CG can prolong the life of the CL in the rhesus monkey (Wilks and Nobile, 1983) and in women (Van de Wiele *et al.*, 1970). Immunization against CG prevents the establishment of pregnancy and 'rescue' of the CL in the marmoset monkey (Hearn, 1979) and rhesus monkey (Moudgal *et al.*, 1971). In early pregnancy, during luteal rescue, CG concentrations in the peripheral circulation double every 2–4 days in the marmoset monkey (Hearn *et al.*, 1988a), rhesus monkey (Atkinson *et al.*, 1975) and in women (Braustein *et al.*, 1973). The ability of CG to stimulate progesterone production by luteal cells *in vitro* has been demonstrated in the marmoset monkey (Webley *et al.*, 1989a), rhesus monkey (Stouffer *et al.*, 1977) and human (Richardson and Masson, 1980) and after perfusion of the marmoset CL *in vivo* (Hearn and Webley, 1987; Webley and Hearn, 1987).

The bovine embryo also secrets a luteotrophic message. It was first identified when plasma progesterone concentrations were found to be considerably higher on day 10 of pregnancy, rather than day 10 of the cycle (Lukaszewska and Hansel, 1980; Hansel, 1981). *In vitro* studies have shown that homogenates from day 18 embryos stimulate progesterone production by bovine luteal cells (Beal *et al.*, 1981), and day 14 embryos in culture secrete a substance which stimulates progesterone production by rat granulosa cells (Plante *et al.*, 1987). The substance with the luteotrophic activity was found to have a molecular weight of <10 000 and be heat-labile and absorbed by dextran-coated charcoal (Hickey and Hansel, 1987). In a more recent report (Hansel *et al.*, 1989), it was suggested that the substance was like platelet-activating factor. As described previously, PAF has also been identified as an embryo message in women (O'Neill, 1985), but the report that PAF may have a luteotrophic action in women, since it increases progesterone production by human granulosa cells in culture (O'Neill, 1987), has yet to be confirmed.

PGE_2 and PGI_2, secreted by the embryo, have been suggested as possible luteotrophic agents in the sheep (Pratt *et al.*, 1977; Bazer *et al.*, 1981; Silvia *et al.*, 1984). PGE_2 dramatically increases in the uterine plasma of ewes on days 13 – 14 of pregnancy (Silvia *et al.*, 1984). In a review, Bazer *et al.* (1986), question the necessity for a luteotrophic agent in the pig as the CL can be maintained after hysterectomy as long as basal LH is available. However, since two or more embryos are required for the establishment of pregnancy, they suggest that in the pig, and also possibly in the cow, PGE_2 may play a role in luteostasis.

(b) Anti-luteolytic messages

Rather than a luteotrophic message, the embryos of the sheep, cow and pig secrete messages which are anti-luteolytic. That is they prevent $PGF_{2\alpha}$, secreted by the uterus, from reaching the corpus luteum and causing luteolysis.

In the pig, it has been proposed that oestrogens secreted by the blastocyst are responsible for the prevention of luteal regression and the establishment of pregnancy. Oestrogens are secreted by the pig blastocyst from day 10 of gestation (Flint *et al.*, 1979; Gadsby *et al.*, 1980; Stoner *et al.*, 1981; Bazer *et al.*, 1986) and the CL can be maintained in pseudopregnancy by injections of oestradiol. It would appear that blastocyst oestrogens do not inhibit uterine production of $PGF_{2\alpha}$ but instead cause a redirection of the release of $PGF_{2\alpha}$ by the uterus so that the amount released into the uterine vein is reduced (Bazer *et al.*, 1982).

In the sheep, the blastocyst secretes a variety of proteins during *in vitro* culture, but two-dimensional polyacrylamide gel electrophoresis (PAGE) showed one major product of interest. This was subsequently purified and termed 'trophoblastin' (Martal *et al.*, 1979), then ovine trophoblast protein 1 (OTP-

1) (Godkin *et al.*, 1982), but is now termed trophoblast interferon (tIFN) (H.J. Stewart *et al.*, 1987; R.M. Roberts *et al.*, 1992). Its antiluteolytic ability to extend the lifespan of the CL was demonstrated by infusing tIFN (or conceptus secretory proteins) from a day 16 embryo into the uterus of non-pregnant ewes and extending the inter-oestrus interval (Godkin *et al.*, 1984; Vallet *et al.*, 1988). Trophoblast interferons have been shown to act directly at the endometrium rather than at the CL and prevent luteal regression by inhibiting the ability of oxytocin and oestradiol to induce $PGF_{2\alpha}$ production (Bazer *et al.*, 1986; Vallet *et al.*, 1988). Both ovine conceptus secretory proteins and recombinant bovine α_1-interferon decrease endometrial oxytocin receptor concentration and suppress $PGF_{2\alpha}$ release following treatment with oxytocin in cyclic ewes and inhibit the formation of endometrial oxytocin receptors in progesterone-treated ovariectomized ewes (Vallet and Lamming, 1991). In recent studies, homology between OTP-1 and α-interferons has been described (Imakawa *et al.*, 1987; H.J. Stewart *et al.*, 1987; Charpigny *et al.*, 1988; Flint *et al.*, 1988) and the characteristics of these compounds compared. This has shown that, in addition to its antiluteolytic action, trophoblast interferons have potent antiviral activity (Pontzer *et al.*, 1988), which may be important in early pregnancy. That ovine tIFN acts locally was shown by measuring antiviral activity and endometrial oxytocin receptor concentrations in ewes made unilaterally pregnant by previously transecting and isolating one uterine horn from the remaining horn and body of the uterus. Under these conditions, tIFN concentrations were high and oxytocin receptor concentrations low in the pregnant horn, whereas the reverse occurred in the non-pregnant horn (Lamming *et al.*, 1991). Thus, by inhibiting endometrial receptor formation, second-messenger responses to oxytocin are precluded, preventing the luteolytic pulses of $PGF_{2\alpha}$ (Flint *et al.*, 1992). Further studies with unilaterally pregnant sheep showed that, while basal $PGF_{2\alpha}$ levels were high in blood draining the pregnant horn, there was little $PGF_{2\alpha}$ response to a bolus injection of oxytocin, whereas in the non-pregnant horn, luteolytic type $PGF_{2\alpha}$ pulses occurred (Payne and Lamming, 1992). This demonstrates the local action of trophoblast interferon in the pregnant horn, which does not require a change in systemic levels of ovarian steroid hormones. The administration of recombinant bovine α_1-interferon to cyclic ewes by both systemic and intrauterine routes inhibits prostaglandin $F_{2\alpha}$ secretion and extends CL lifespan (Parkinson *et al.*, 1992).

In the cow, oxytocin- and oestradiol-induced $PGF_{2\alpha}$ production is also inhibited by the presence of the conceptus (see Thatcher *et al.*, 1985). Proteins secreted by the cow embryo in culture extend the life of the CL (Knickerbocker *et al.*, 1986; Helmer *et al.*, 1989a,b). The protein, termed bovine trophoblast protein 1 (BTP-1) is similar immunologically to OTP-1. The two proteins may be biologically active in both species as interspecies transfer of trophoblastic vesicles altered the lifespan of the CL of the recipient species (Heyman *et al.*, 1984; Martal *et al.*, 1984). BTP-1 has been shown to prevent luteolysis by acting at the endometrium to inhibit the production of prostaglandin by inducing synthesis of an intracellular inhibitor of $PGF_{2\alpha}$ synthesis (Helmer *et al.*, 1989b). Infusion of bovine α-interferon into the uterus of cyclic cows delayed luteolysis suggesting that, as in the sheep, an interferon-like molecule may be important in the establishment of pregnancy (Plante *et al.*, 1988).

It can be suggested that CG secreted by the primate embryo should also be termed an anti-luteolytic as well as or rather than a luteotrophic message. If CG is able to maintain the life of the CL in early pregnancy by preventing the luteolytic action of $PGF_{2\alpha}$ (Knobil, 1973; D.T. Baird, 1985; Hearn and Webley, 1987) or, alternatively, its pro-

duction, then the only difference in the required action for the embryo message, between the primate and the above species, is the source of the luteolytic agent.

6.3.3 TIMING OF THE EMBRYO MESSAGES

The timing of events is, however, rather different between primate and non-primate species, although in all species the timing of release of the embryo message is critical. In the primate, CG is secreted at the time of implantation. Implantation begins on days 8–9 in women (Lenton and Woodward, 1988), the rhesus monkey and baboon (Hendrickx and Enders, 1980) and rather later on days 11–12 in the marmoset monkey (Hearn, 1980; H.D.M. Moore *et al.*, 1985; Smith *et al.*, 1987). Although CG cannot usually be detected in the peripheral circulation until some 3 days later (Hearn, 1986), culture of embryos *in vitro* shows a clear secretion of CG from the time of attachment in the human (Fishel *et al.*, 1984) and marmoset monkey (Hearn *et al.*, 1988a).

Comparison of progesterone production by luteal cells from the marmoset monkey during short-term incubation *in vitro* showed differences between cells taken from pregnant and non-pregnant animals on day 14 after ovulation (Webley *et al.*, 1989a). Progesterone production by luteal cells from non-pregnant animals responded to stimulation by luteotrophic agents, whereas the luteal cells from pregnant animals were unresponsive; control production of progesterone was high and already equivalent to the levels stimulated by the treatments (Figure 6.8).

This suggested that on day 14 (just 2 days after implantation, which occurs on days 11–12) a message secreted by the embryo may have affected the CL, providing an endogenous stimulus. In the marmoset, the secretion of CG begins at the time of attachment of embryos in culture, although measurement of CG in the peripheral circulation does not rise above background levels until day 17 (Hearn *et al.*, 1988a). If CG is not detectable for 3–4 days after embryo attachment is it CG or another message received by the CL within 2 days of attachment? In women, using very sensitive assays for hCG and accurate extrapolation of the hCG curve, an increase in levels could be detected within 2 days (Lenton and Woodward, 1988).

If CG is secreted into the peripheral circulation at the time of implantation, in sufficient quantities to reach the CL, there may be no need to postulate a role for other embryo messages in the rescue of the CL during early pregnancy in the primate. Indeed the ability of a pre-implantation embryo message to reach the CL via the peripheral circulation is questionable. However, while administration of exogenous CG can extend the life of the CL, it can do so for only a few days (diZerega and Hodgen, 1980; Ottobre *et al.*, 1984). This could result from the CL becoming desensitized to repeated injections of hCG or the involvement of another luteotrophic agent in the rescue of the CL (discussed by Moudgal, 1984; Richardson, 1986). In the marmoset monkey, luteal cells taken from pregnant animals on day 20 after ovulation had a lower basal production of progesterone than luteal cells from day 14 pregnant animals and the cells responded to stimulation by luteotrophic agents, whereas the day 14 luteal cells did not (Webley *et al.*, 1989a). CG concentrations in the peripheral circulation of the marmoset are undetectable on day 14 whereas on day 20 levels are detectable and rising rapidly (Hearn *et al.*, 1988a). Consequently, if CG could influence the CL before it was detectable in the peripheral circulation the luteal cell response must alter between days 14 and 20 or pregnancy when the CL of pregnancy is established. Alternatively, the embryo may secrete a different message to 'rescue' the CL before CG reaches sufficient concentration in the circulation to take over the luteotrophic role.

In the sheep, the embryo secretes the anti-

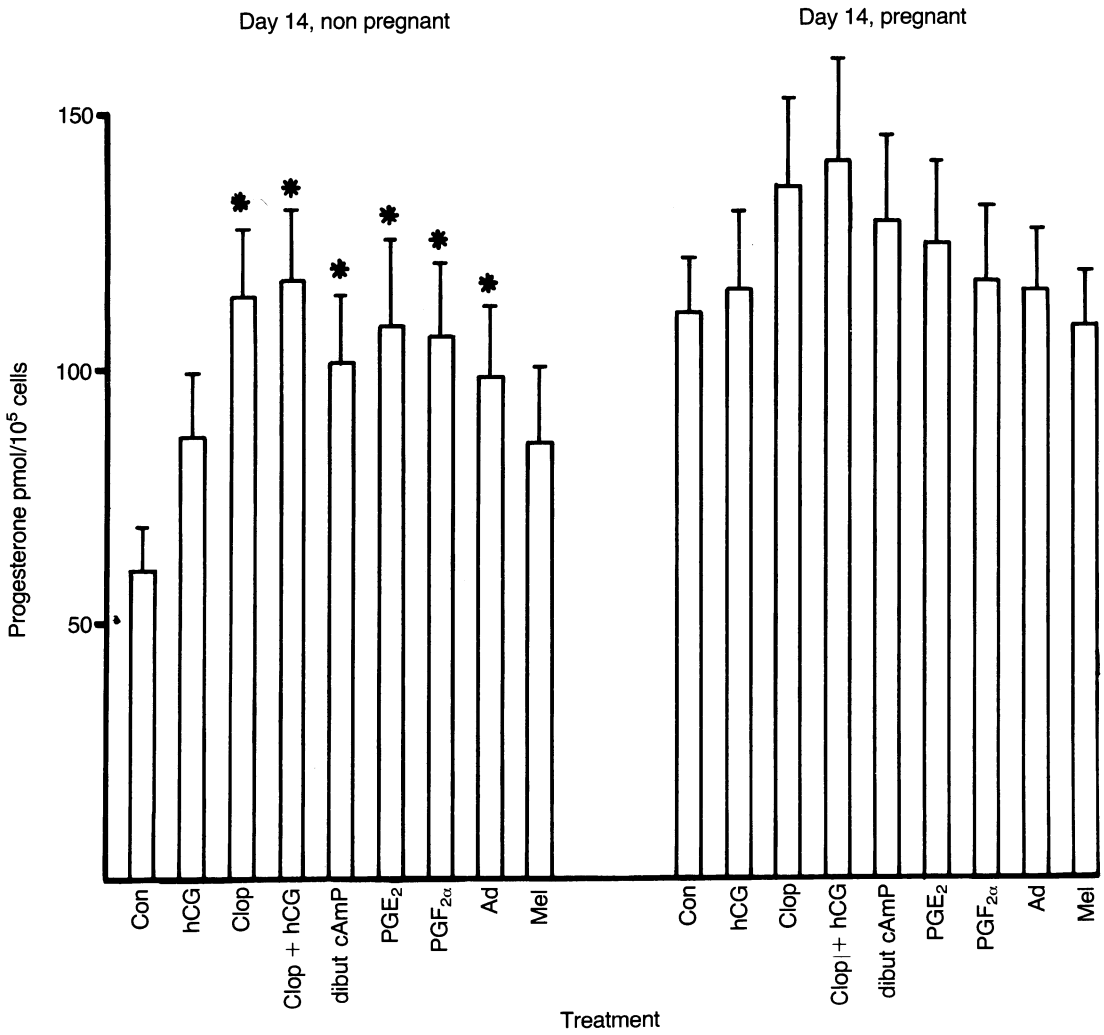

Figure 6.8 Progesterone concentrations (mean ± SEM) produced by dispersed luteal cells from non-pregnant and pregnant marmoset monkeys on day 14 after ovulation after incubation for 2 h with hCG, cloprostenol (Cp) ± hCG, dibutyrl cAMP (db), PGE_2 (E_2), $PGF_{2\alpha}$ ($F2_{2\alpha}$), adrenaline (Ad) and melatonin (mel). C=control. *$P<0.05$ compared with control values (Duncan's multiple range test).

luteolytic protein OTP-1 for a short period at the beginning of pregnancy between days 12 and 21 (Godkin *et al.*, 1982). It must be produced by day 12/13 as the CL begins to regress at this time; if an embryo is transferred to a non-pregnant ewe on day 15 it is too late and luteal regression occurs (Moor and Rowson, 1966). Once luteolysis has been prevented, the life of the CL continues after day 21 when the embryo stops secreting OTP-1. In the cow, the embryo also secretes BTP-1 for a limited period from days 16–24 of pregnancy. Proteins secreted by the bovine embryo in culture on days 16 to 18 extended the life of the CL and inter-oestrus interval when administered into the uterine lumen of cyclic cattle on days 15–21 after oestrus (Knickerbocker *et al.*, 1986).

Primates differ from the domestic species in the timing of secretion of the embryo mess-

Table 6.6 Comparative rates of pre-implantation development in mammals

Species	*Two-cell*	*Eight-cell*	*Morulla*	*Blastocyst*	*Uterine entry*	*Implantation*	*Gestation length*
Mouse	1[a]	2	3	3·5	3·5	4	20
Rat	1	2	3	3·5	3–4	5	22
Rabbit	2	2·5	3	3	3	7	32
Cat	3	3·5	4–5	5–7	4–8	13–14	62
Dog	4	6	7	8	7–8	17	63
Ferret	2	4	5	6	5	15–16	41
Pig	1	2.5	4	5	2.5	11–20	114
Sheep	1	2·5	4	6	4	15–16	150
Cow	1	3	4–6	7–9	4	30–35	280
Horse	1	3	4	6	4	49–63	340
Marmoset	1	4	7–8	8–9	4	10–12	144
Rhesus	2	3	4–5	5–6	3	8–9	165
Baboon	1.5	3	4	5–6	4	8–9	180
Human	1.5	2.5	3	4–6	2–3	8–9	266

[a]Days after fertilization (from the literature).

age. In primates, CG is secreted at the time of implantation, whereas in domestic species the embryo signals are sent to the mother before there is intimate contact at implantation. In the pig, oestrogens are secreted by the blastocyst from day 10, production is decreased between days 13 and 14 then increases between days 15 and 25–30, while attachment of the embryo occurs on day 18 (Gadsby *et al.*, 1980; Bazer *et al.*, 1986). In the sheep, OTP-1 is secreted from day 12 (Godkin *et al.*, 1982), whereas attachment occurs on day 16 and in the cow BTP-1 is secreted from day 15 (Knickerbocker *et al.*, 1986) and attachment occurs between days 25 and 30. The difference between primates and domestic species is that the primate embryo message must reach the ovary via the circulation, whereas the embryo messages secreted by the embryo of domestic species acts at the uterus to prevent release of uterine $PGF_{2\alpha}$, so close contact between the embryo and the maternal circulation may not be required.

This part finishes the discussion of interrelationships between embryo and mother. The signal for pregnancy has been established. It is now important to discuss how the embryo and fetus develop within the maternal organism.

6.4 RATES OF DEVELOPMENT

6.4.1 PRE-IMPLANTATION PERIOD

Prenatal development includes the period from fertilization to birth and is characterized by a series of external and internal morphological changes that are similar in many mammalian species. However, the time course of cleavage, organ formation and fetal growth vary both within and between different species. Following fertilization, the embryo undergoes cleavage, the process of cellular division without cellular growth. Cleavage continues until implantation. At this time cell size has been reduced to the approximate size of the normal somatic cells characteristic of the species. In general, the laboratory species have shorter gestation periods than domestic species and slightly greater rates of cleavage (Davies and Hesseldahl, 1971; Brinster, 1974; McLaren, 1974) (Table 6.6).

In most species, the embryo undergoes cleavage while in the oviduct and does not pass into the uterus until the late morula to early blastocyst stage. The oviductal transport of the embryo during this period prior to implantation is a complex process exhibiting marked species variability. Muscular activity, ciliary movements and tubal fluid currents contribute to the propulsion of the embryo to varying degrees. Passage of the embryos through the oviduct takes 3–4 days in most mammals (Wimsatt, 1975), but it can be as short as 24 h (opossum, Hartman, 1928) or 48 h (pig, Hunter, 1974) or as long as 8–10 days (dog, Holst and Phemister, 1971) (Table 6.6).

Blastocysts undergoing cleavage move through different portions of the oviduct at variable rates. Rabbit oocytes (or ova) reach the junction of the ampulla and isthmus as quickly as 6 min after ovulation, but the remainder of the time in the oviduct is spent in slow transport through the isthmus prior to entry into the uterus (Bodkhe and Harper, 1973). In contrast, the ova of mice and rats are transported rapidly through the ampulla and then detained at the isthmoampullar junction for 24 h. Transport through the isthmus is again rapid with a 30-h delay at the uterotubal junction (Humphrey and Martin, 1969). This pattern of rapid movement to the isthmoampullar junction, a delay at the site of fertilization, then rapid movement to the tubouterine junction with a second delay, appears to predominate in most mammals (D.H. Anderson, 1927; Blandau, 1961). A third type of oviductal transport is exhibited by pig and cow ova, which spend most of the time above the isthmo ampullar junction and then move rapidly through the isthmus into the uterus (Aref and Hafez, 1973; Blandau, 1973). Studies in human (Croxatto and Ortiz, 1975) and non-human primates (Eddy *et al.*, 1975) indicate that tubal transport in these species is similar to the third mechanism.

The first overt sign of cellular differentiation occurs with the development of the blastocoele, which usually coincides with the arrival of the embryo in the uterus. During the blastocyst stage, the zona pellucida is lost and the embryo breaks free (hatches). There is a marked increase in the total size and often a change in shape of the embryo during this stage, mainly due to the enlargement of the blastocoele. Considerable species variability is observed in both the rate and magnitude of blastocyst expansion following blastocoele formation. While marked distension of the blastocyst is common to the primate, rabbit, dog, cat and most livestock species (Brinster, 1974; G.B. Anderson, 1977; Noden and deLahunta, 1985), minimal expansion takes place in the mouse, rat and guinea pig (Hafez, 1972).

Essentially two cell types become recognizable in the blastocyst. Those that accumulate at one pole become slightly larger and constitute the embryonic disc (inner cell mass, blastodisk), from which the embryo will develop. The cells on the periphery of the blastocyst are trophoblast cells, which facilitate the absorption of nutrients early in development and later participate in the formation of extraembryonic membranes, which contribute to the formation of the placenta. In domestic animals the trophoblast cells overlying the embryonic disc degenerate or shift peripherally and the blastodisc becomes exposed on the surface of the blastocyst. In most primates the embryonic disc remains beneath the trophoblastic cover and is referred to as the inner cell mass (Noden and deLahunta, 1985).

Implantation is a transitional stage in early pregnancy during which the blastocyst assumes a fixed position within the uterus. The steps involved are species dependent but usually include apposition and adhesion of the blastocyst to the surface of the uterine epithelium followed by penetration of the epithelium by the blastocyst (Boving, 1963; Enders, 1972). The loss of the zona pellucida in relation to the time of implantation varies with the species and mechanism of implan-

tation. In animals in which implantation is eccentric (rat, mouse, hamster) or interstitial (bats and primates), the zona is lost before implantation is initiated. The blastocysts of animals with centric implantation (rabbit, pig, sheep, cow, horse, dog and cat) expand and implant with the extracellular coat(s) surrounding the trophoblast still intact (Wimsatt, 1975). Detailed morphological descriptions of implantation have been provided for a few non-human primate species, including the rhesus monkey (Enders *et al.*, 1983), baboon (Tarara *et al.*, 1987) and marmoset (Smith *et al.*, 1987); in contrast, the availability of comparable human material for evaluation is limited to the study of Hertig *et al.* (1956).

6.4.2 ORGANOGENESIS

Organogenesis encompasses the period of embryonic development from the establishment of the primitive streak, which marks the future longitudinal axis of the embryo, until palate closure and ossification of the humerus. During this time the three germ layers are reshaped to form the embryo proper by a process combining growth with cell and tissue movements. The major changes during the period of organogenesis are elongation of the body, subdivision of the body into head, trunk and tail, development of the appendages, and separation of the embryo from the extraembryonic structures. By the end of this period the shape of the embryo has changed greatly and the major features of the external body form are recognizable. The morphological features of human embryos during organogenesis have been well described (see O'Rahilly and Müller, 1987). A number of non-human primate species have also been the subject of developmental staging, which utilizes size and age criteria, in addition to morphology, to define this period of prenatal growth (Hendrickx, 1971; Gribnau and Geijsberts, 1981; Butler, 1983; Binkerd and Hendrickx, 1984). While the rudiments of the various organ systems conform to generalized, basic plans in the embryos of all vertebrate species, the temporal aspects of organ formation are species specific (Table 6.7).

Studies in mice have shown that up to the time of primitive streak formation cell proliferation in the embryo remains very slow. Measurements of tissue and cell volume in mouse embryos (at 6.5 days) show a cell population of only 500–600 cells (Snow, 1976). With the onset of gastrulation (primitive streak formation) the number of cells has increased to 15 000 within a 24-h period, requiring a doubling of the previous proliferation rate (Snow, 1977). In other species there is also an acceleration in growth rate during primitive streak formation. It has been estimated that human (Boyd and Hamilton, 1964; Langman, 1975; K.L. Moore, 1977) and pig (Patten, 1953) embryos increase some 40-fold in size in 4 days, while the baboon (Hendrickx, 1971) increases about 16-fold in 3 days. If this change in volume is produced entirely by an increase in cell number (rather than changes in cell volume or intercellular spaces), then cell proliferation rate has accelerated to about the same extent as in the mouse, i.e. doubled (Snow, 1981).

From the neural plate stages through organogenesis, various regions of the embryo establish their own growth profiles characteristic of the organs into which they will develop. In the mouse, the high cell proliferation rate of gastrulation stages is not maintained throughout the embryo during this period (Snow, 1986). Although more attention has been devoted to fetal growth in humans, a considerable body of information has recently been accumulated on the corresponding age, stage and length as well as the growth of a few organ systems in the human (O'Rahilly and Müller, 1986).

6.4.3 FETAL PERIOD

The fetal period is characterized by maximum growth and further development of the

Table 6.7 Comparative times of development during organogenesis (days)

	Mouse	*Rat*	*Rabbit*	*Guinea pig*	*Cat*	*Dog*	*Pig*	*Sheep*	*Cow*	*Horse*	*Marmoset*	*Macaque*	*Baboon*	*Human*
Primitive streak	7[a]	8.5	6.5	13.5		15	12	13–14	18	14	25–28	16–18	16–18	13–15
First somite	8	9.5	8.5	14.5	13	16	14–15	15	19		55–59	20–21	22–24	19–21
Anterior neuropore closure	9	10–11	9	15.5							52–60	24–26		23–26
Posterior neuropore closure	9	11	9	16.5		17	16	21	23	18		27–28	27–29	26–30
Anterior limb bud	9.5	11	10–11	16.5	18	22	16–17	20	24	26	51–70	27–29	28	26–30
Posterior limb bud	10–11	11–12	10.5	17.5	19	22	17–18	21	26			28–29	28–30	28–32
Four branchial arches	10.5	12	10.5	17.5	17	25	15–17	21	26		51–67	28–29	28–30	
Eye pigmentation	11		14	17.5	21	25–28	20–21	25	30	36	66–83	31–32	30–32	37–42
Digit separation distally	14.5	16–17	17	27	24	35		34	45			45–47		56–58
Eyelid fusion	16	18	20	35	31	40	50	43	60	63		45–46	46–48	46–48
Palate fusion	15	16–17	19–20	33	32	33	34.5	38	56	47	8	45–47	46–48	56–58

[a]Day of mating is considered as day 0 of gestation. (From the literature.)

organs and systems established during the embryonic stage. Absolute prenatal growth continues throughout gestation while relative growth (percentage increment change) begins to decrease about midway in gestation. Fetal crown–rump length and weight are the indices most consistently correlated with gestational age in growth assessment studies. While crown–rump length reflects the growth of the head and vertebral column, weight is determined mainly by the developing muscles and other soft tissues (Bergin *et al.*, 1967).

Figure 6.9 shows the growth curves for length and weight for a normally developing human fetus.

There is a steep linear relationship between fetal length and gestational age after the second month with an average increase in sitting height (crown–rump) of 1.5 mm/day (Patten, 1968). Maximal linear growth during gestation occurs in the sixth and seventh months and decelerates during the last 2 months of the fetal period (Scammon and Calkins, 1929). The relationship between weight and gestational age is more curvilinear; from the time of fertilization to birth the increase in weight is approximately 6 billion times (Lowrey, 1978). The 'shape' of the growth curves for several species, including the pig (Ullrey *et al.*, 1965), rhesus monkey (van Wagenen and Catchpole, 1965), baboon (Hendrickx, 1971), cow (Evans and Sack, 1973; Eley *et al.*, 1978) and rat (Angulo and Gonzalez, 1932), is similar to that of the human.

Intraspecies comparisons of fetal growth have been made by quantitative descriptions of these two parameters as a function of gestational age. Haase's rule describes the linear

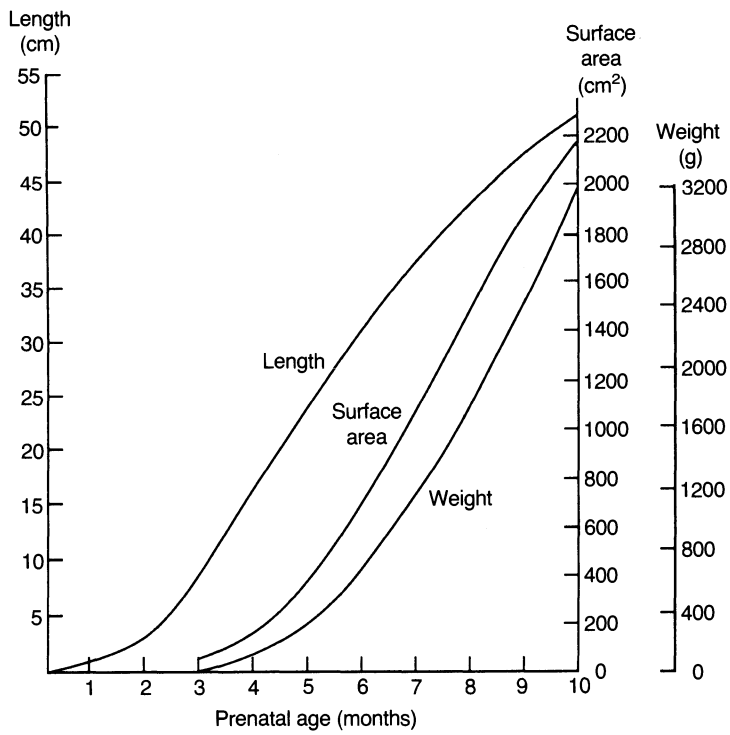

Figure 6.9 Prenatal growth curves for the human fetus from time of conception to birth, length (cm, crown–heel), surface area of the body (cm^2) and weight (g).

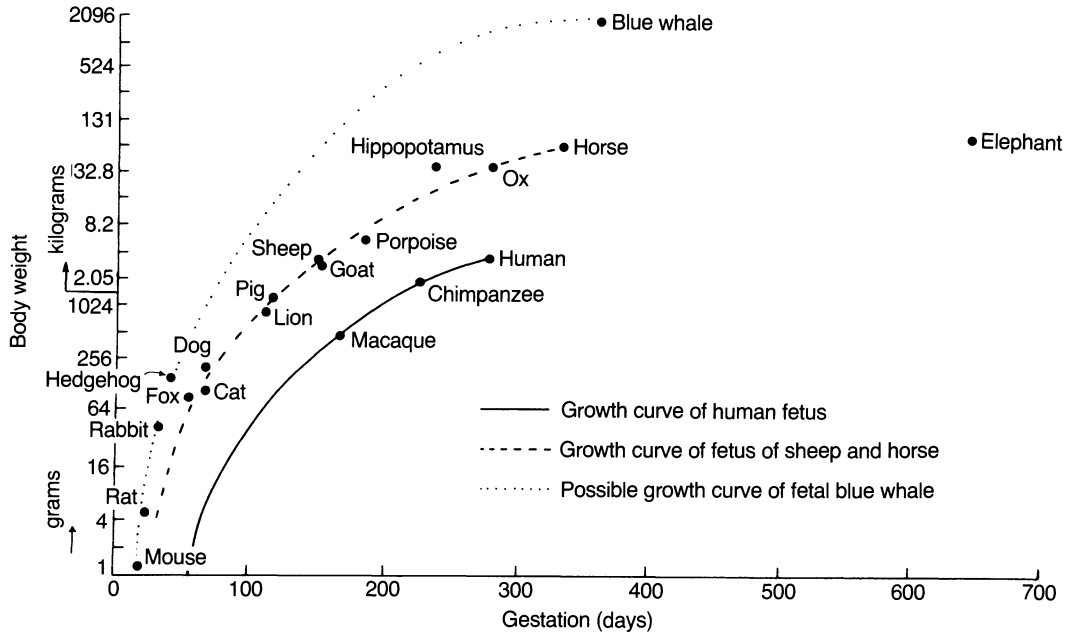

Figure 6.10 Birth weights of 20 species and their lengths of gestation. The curves show that the species can be classified into those that grow very fast (. . .), fast (- - -) and slow (——). (Reproduced with permission from McCance & Widdowson, 1986.)

relationship between the length of the fetus of many species and gestational age during the second half of pregnancy (Spencer and Coulombe, 1965). It has been determined that the fetal weight of many species is a cubic function of time after the appearance of the primitive streak (corresponding to the time when placental function becomes established). The equation is $W = a(t-t')^3$ where W is the weight of the fetus on any given gestational day, a is a constant expressing the rate of supply of nutrients per unit of fetal surface area, t is the length of gestation, and t' is the lag period of growth prior to placental function (Page *et al.*, 1981). For man the value of a is 0.24×10^6 and for large mammals the value is considerably larger (e.g. 16 times as great in the cow). The rate of growth for most mammals is enormous initially and progressively slows as pregnancy progresses. For example, in the first month after fertilization, the human zygote increases a million times in weight, whereas in the last month of gestation the rate of increase is 0.3. A decrease in the value of a toward the end of gestation will result in intrauterine growth retardation (Page *et al.*, 1981).

The rate of growth soon after conception sets the pace for the subsequent growth *in utero* and thus the weights at birth for similar lengths of gestation. The size at birth is the product of the rate and duration of growth. The relationship between these variables is different among mammals, some achieving final size quickly by rapid growth, others at a slower pace for a longer period *in utero*. Figure 6.10 shows the weights at birth of 20 species plotted against their length of gestation.

The points appear to fall on three curves which describe three classes of mammals: very fast, fast and slow growers (McCance and Widdowson, 1986). As seen in this

figure, the small rodents (mouse, rat) and the rabbit as well as the fetal whale grow extremely rapidly throughout gestation. In contrast, man, the macaque and the elephant grow more slowly in the early stages, which results in lower weights after similar times in the uterus. An intermediate growth rate is exhibited by the cat, pig, dog, guinea pig, goat, ox, sheep, porpoise, hippopotamus and horse. These differential growth rates and gestational lengths result in different sizes at birth. Thus, the newborn hippopotamus is larger than the newborn human baby because it grows faster. The human fetus grows much larger than the rat before it is born because it stays in the uterus longer (McCance and Widdowson, 1986).

The prenatal growth period is characterized by a progressive alteration of form and proportions, both externally and internally. Diversity of form is acquired through differential rates of growth operating in various regions of the fetus and in definite directions. While the absolute rates may vary among individuals of any species, the ratios existing between the growth rates of different parts of the body in that species are relatively constant. The emergence of limbs from initial bud-like swellings and the early form changes during the development of the brain are visual examples of the progressive modelling of external and internal form by differential growth (Arey, 1974).

Assessment of intrauterine growth in humans using radiographic, anthropometric and ultrasonic methods has made a major contribution to a better understanding of the principle of fetal growth in relationship to gestational age (P.R.M. Jones *et al.*, 1986; Meire, 1986). The establishment of growth curves generated by clinical ultrasound observations and measurements of various fetal dimensions provides information on gestational staging, somatic growth and functional maturity in addition to diagnosis and management of fetal growth disorders including intrauterine growth retardation (Birnholz, 1986; Bowie, 1988; Reece *et al.*, 1989). Assessment of intrauterine growth in non-human primates has also been advanced by the recent use of ultrasonography in monitoring pregnancy in rhesus and cynomologus monkeys (Tarantal and Hendrickx, 1988a).

In humans, the skeleton and musculature grow rather slowly until the last two fetal months, during which time they each show accelerated growth to constitute from 15 to 25% of the total body weight of the newborn. The central nervous system, on the other hand, makes up a larger portion of the body weight in the young embryo (25% in the second month) than at birth (15%). As a whole, the viscera decrease slowly and steadily in relative weight after the first two embryonic months. In the second prenatal month these organs constitute about 15% of the total body weight, which is reduced to approximately 9% at birth (Arey, 1974). Although each fetal organ has its characteristic growth curve, the individual organs generally increase rapidly to a maximum relative size during the early fetal period and then decrease with age even to maturity (Arey, 1974). Recent growth curves for the spleen, kidney and liver as well as foot length in human fetuses have been correlated to fetal parameters of maturity, including gestational age, crown–rump length/weight (Ferreira *et al.*, 1990, 1992; Mandarim-de-Lacerda, 1990; Sampaio and Aragao, 1990).

6.4.4 FACTORS AFFECTING PRENATAL GROWTH

Fetal growth rate and size at birth are affected by a combination of constitutive and environmental factors.

(a) Constitutive factors

While the fetal and maternal genomes dictate the growth potential of the fetus, adequate amounts and successful transfer of substrate are needed to achieve this potential. The ulti-

mate definition of fetal size during normal pregnancy is a result of regulation of this genetic and environmental blueprint by hormones and growth factors.

(b) Genetic factors

Genetic factors have an important influence in determining fetal growth rates characteristic of different species and contribute to the normal biological variation in size within a species. Non-chromosomal instructions appear to be especially important in the earliest stages of development immediately after fertilization, which are heavily influenced by the maternal genotype through the cytoplasmic genetic information. At fertilization the sperm is only made up of chromosomes with negligible amounts of other material, whereas the maternal contribution embodies the full cytoplasmic material as well as chromosomes (D.F. Roberts, 1981). Thereafter, differentiation and development proceed as a complex of gene-controlled synthetic processes resulting largely from switching on and off the activity of particular fetal genes at specific times.

The existence of genetic influence on human fetal growth is evidenced by the fact that infant birth weight is grossly affected by genetic disease, both single gene and chromosomal (Rimoin and Graham, 1989). Infants with fibrocystic disease and galactosaemia as well as those with Down's syndrome, trisomy 13 and Turner's syndrome, for example, are of reduced mean birth weight. Since only the XO aberration is consistently associated with reduced growth rate in humans, the genetic control of intrauterine growth is believed to involve loci widely distributed through the autosomes (D.F. Roberts, 1981).

Fetal genotype has been estimated to account for approximately 20% of birth weight variation in humans (Polani, 1974). Male genotype is associated with increased birth weight, with males averaging 150–200 g more than females at term (H.C. Miller and Merritt, 1979). This difference in fetal size is not apparent in early gestation, but rather appears to be dependent on increased growth in the third trimester (Thomson *et al.*, 1968). Clearly, genetic influences play a part in some of the variation noted in birth weights among different ethnic groups. Although some of these differences may be explained by environmental factors, a number of studies describe striking ethnic differences in birth weight, irrespective of socioeconomic status (Barron and Vessey, 1966).

Thus, the contribution of the fetus's own genes in determining its size at birth is small compared with the contribution of maternal factors – both environmental and genetic. While in early pregnancy fetal genetic control is dominant and determines relatively narrow limits of variability in patterns of fetal growth, maternal and placental constraints as well as hormonal and growth factor stimuli assume increasing importance and give rise to greater variability in fetal growth later in pregnancy (Liggins, 1982). After birth such strong prenatal influences upon growth are gradually neutralized. The individual's own genes begin to reassert their influence, and post-natal growth moves firmly in a direction determined genotypically (R.S. Wilson, 1976).

(c) Hormones

The translation of genetic information to actual growth requires regulation by fetal hormones which mediate utilization of available substrate. Insulin plays a significant role in this regulation of fetal growth. Human fetuses either deficient in insulin due to infant diabetes or pancreatic agenesis or resistant to insulin, as in leprechaun syndrome, are small in size (D'Ercole and Underwood, 1986). The level of serum insulin in the human fetus is thought to reflect autonomous fetal pancreatic insulin secretion, since the

placenta is impermeable to insulin throughout pregnancy (H. Wolf *et al.*, 1969). Various clinical syndromes associated with excessive fetal growth, such as diabetes, Beckwith–Wiedemann syndrome, nesidioblastosis, insulinoma and erythroblastosis, have associated hyperinsulinism (D.E. Hill, 1976). Although the mechanism of action of insulin in the induction of overgrowth is uncertain, some evidence suggests that it can enhance insulin-like growth factor 1 (IGF-1) secretion (Gluckman, 1989).

A number of animal models support this growth-promoting role for insulin. Rat fetuses injected near term with subcutaneous insulin showed evidence of macrosomia and increased glucose utilization (Picon, 1967). Surgical removal of the pancreas in lambs (D.E. Hill, 1976), alloxan injection into fetal rabbits (P.G.R. Harding *et al.*, 1975) and streptozotocin injection into fetal monkeys (Cheek and Hill, 1975) have all resulted in hypoinsulinaemic, growth-retarded fetuses. *In vivo* and *in vitro* animal studies suggest that insulin is permissive for fetal growth and that its growth-stimulatory actions in the fetus are mediated by its effects on carbohydrate, protein and fat metabolism, rather than through direct stimulation of cellular proliferation (D'Ercole and Underwood 1986).

The effect of growth hormone on fetal development is not well understood. Human infants with familial isolated growth hormone deficiencies and those with high levels of inactive growth hormone are generally of normal weight, although their length may be diminished at birth (Laron and Pertzelan, 1969). That growth hormone may not be crucial in humans is also supported by the fact that newborn infants with anencephaly and congenital absence of the pituitary are not small in size (D'Ercole and Underwood, 1986). The effect of interference with hypothalamic and pituitary development and function seems to be species specific, i.e. fetal decapitation *in utero* has minimal effect on fetal size in the rat, mouse, hamster, pig and rabbit, while in the calf and lamb significant differences were seen following both naturally occurring absence of and surgical destruction of the fetal pituitary (Liggins, 1974; Jost, 1979). Neuronal growth in rats appears to be enhanced by administration of exogenous growth hormone (Honnebier and Swaab, 1974), although somatic growth is unaffected.

There are conflicting results with respect to the direct effect on somatic growth of the adrenal steroids. An increasing number of studies in humans (Taeusch, 1975) and experimental animals (de Lemos, 1976; Frank and Roberts, 1979) have demonstrated that exogenous glucocorticoids have a negative effect on fetal growth. Experimentally adrenalectomized lambs (Drost and Holm, 1968), rats (Jacquot, 1959) and monkeys (Mueller-Heubach *et al.*, 1972) have birth weights within normal limits. However, in some cases of idiopathic adrenal hypoplasia in humans, birth weights are slightly reduced (Liggins, 1974). Depending on the cell type, glucocorticoids either inhibit or stimulate the proliferation of fetal cells in culture (Jimenez de Asva *et al.*, 1977). The role of thyroid hormone appears to vary in different species. Fetal hypothyroidism is associated with growth retardation in some species, but not in others, including man. Experiments in sheep with placental lactogen raise the possibility that this hormone is also involved in the regulation of fetal growth; however, its role in human pregnancy remains uncertain (Gluckman, 1989).

(d) Growth factors

A number of biologically active substances that stimulate mitosis and/or differentiation have been thought to be important in fetal growth (see reviews by Rizzino, 1987; Mercola and Stiles, 1988). Among the peptides that have been purified, sequenced and studied for their effects on *in utero* growth are nerve growth factor (NGF) (Springer, 1988), epidermal growth factor (EGF) (Burgess,

1989; Marti *et al.*, 1989) and the somatomedins (SMs) (Heyner *et al.*, 1989). Although there is no direct proof of the effects of these factors on fetal growth, they have been implicated in human or animal fetal development by one or more lines of evidence:

1. stimulation of proliferation and/or differentiation of cultured embryonic and fetal cells;
2. identification of growth factor receptors in fetal tissues;
3. synthesis of growth factors by fetal tissues; and
4. circulation of growth factors in fetal blood.

Other factors which have also been well studied include haemopoietic growth factor (Gabrilove, 1989) fibroblast growth factor (FGF) (A. Baird and Walicke, 1989) and transforming growth factors (TGFs) (Rizzino, 1988).

It has been postulated that growth factors exert their effects on fetal development by mediating the actions of classic hormones on target tissues (D'Ercole and Underwood, 1986). There is evidence that EGF and NGF are dependent on thyroid hormones and SM might be dependent on prolactin, placental lactogen, insulin or growth hormone. The effect that growth factors have on embryonic and fetal development is probably a result of local tissue action via autocrine or paracrine mechanisms. The regulatory mechanisms by which these factors influence cell growth and differentiation will be facilitated by future studies of embryos cultured in serum-free media (Rizzino, 1987).

(e) Environmental factors

The major environmental factors which affect prenatal growth in mammals include size, age, parity, nutrition and health of the mother, litter size, duration of pregnancy and placental size and function.

(f) Maternal size, age and parity

The size of the mother is positively correlated with prenatal growth rate in most mammalian species. This effect is much more pronounced than the father's size and is most obvious in animals with long gestation periods and singleton pregnancies. The influence of maternal size is obscured by litter size in polytocous animals (Hendrickx and Houston, 1970).

Although the effect of maternal age on fetal size has not been fully investigated, young mothers tend to have smaller offspring, partially because they continue to grow during pregnancy and they compete with the fetus for available nutrients (Hafez, 1968).

Studies in humans have shown that parity may affect size at time of delivery. The average birth weight increases with increasing order, i.e. the birth weight and dimensions of the firstborn are less than those of subsequent infants (Lowrey, 1978). In lower species, parity primarily affects the level of prenatal mortality rather than fetal growth rates. Experiments in mice and rats indicate a decrease in the size of litters with each successive pregnancy and, hence, with age. This decrease has been attributed largely to higher embryonic loss in older animals since the number of shed ova remains fairly constant after the first pregnancy (Hollander and Strong, 1950).

(g) Maternal nutrition and health

In most polytocous animals in which the total weight of the litter is large relative to the weight of the mother, undernutrition has some effect on birth weight. However, studies in rats and rabbits indicate that restriction of maternal food intake has to be extreme before fetal birth weight is markedly decreased (Campbell *et al.*, 1953; Dawes, 1973). The effect of maternal nutrition is noted only to a minor degree in the higher primate species, including the human. Since

nutrient requirements for the fetus follow a similar trend to fetal growth (very low in early pregnancy and increasing markedly in the last trimester), the effects of maternal over- or undernutrition on fetal growth in all species are operative only in about the last one-sixth of gestation (Rattray, 1977; Page *et al.*, 1981).

The effects of maternal health on fetal growth have largely been provided by the medical literature. The most common identifiable cause of intrauterine growth retardation is maternal vascular disease, such as pre-eclampsia, chronic essential hypertension, collagen vascular diseases and neuropathies, including diabetic neuropathy (Oppe *et al.*, 1957). Vascular disease is thought to lead to a decrease in fetal growth by interfering with uteroplacental blood flow (J.C. Browne and Veall, 1953). Other conditions, including chronic antepartum haemorrhage, diminished cardiac output, cyanotic congenital heart disease and various haemoglobinopathies presumably lead to decreased fetal growth because of decreased ability to transport substrate to the fetus or a decreased availability of oxygen in the maternal vascular component (Resnik, 1978).

Intrauterine infections, including rubella, cytomegalovirus, varicella and herpes infections, have also been associated with growth retardation in the fetus (Nahmias and Visintine, 1976; Young, 1976; Knox, 1978). There is increasing evidence that certain environmental factors (i.e. smoking, alcohol, drugs) also affect intrauterine growth. The adverse effects of maternal smoking on fetal growth have been partially attributed to the impairment of uteroplacental circulation as a result of the vasoconstricting effects of nicotine. Low birth weights and lengths are also associated with prenatal exposure to alcohol, which readily accumulates in, but is slowly eliminated from, the fetus. Recent studies suggest that maternal use of cocaine adversely affects fetal growth through impairing fetal oxygenation by catecholamine-induced vasoconstriction of the uterine arteries (Kretchmer *et al.*, 1989).

(h) Litter size and duration of pregnancy

In nearly all polytocous species which have been examined, increases in litter size reduce the rate of prenatal growth and thus birth weight. The influence of litter size on fetal size is manifested through a 'local effect' and a 'general effect' (Eckstein *et al.*, 1955). The effect of other nearby fetuses in the same uterine horn is the 'local effect' and that of any fetus anywhere in the uterus is the 'general effect'. The ratio of each factor varies with the species. Studies in guinea pigs, mice, rabbits and humans support a haemodynamic explanation for this phenomenon, i.e. retardation of growth is due to restriction of the channel (i.e. placental exchange) which links mother and fetus rather than to a limitation of maternal nutritional substances or uterine space (McKeown *et al.*, 1976).

The weight of the entire litter relative to maternal weight shows an inverse relationship, with the smaller mammals having rapidly growing, relatively heavier litters as compared with larger mammalian species. Two extreme examples of this are the guinea pig, which has a litter weight of more than 50% of maternal weight, and the polar bear fetus, which weighs 0.3% of mean maternal weight (Gewolb and Warshaw, 1983). Fetal genes may contribute to these interspecies differences in fetal growth rate, i.e. those species reaching maturity earlier in post-natal life are genetically programmed for a rapid rate of prenatal growth, while other mammals, including humans, have a correspondingly lower fetal growth rate and hence a lower fetal–maternal weight ratio (McKeown *et al.*, 1976).

These litter effects are determined in part by the duration of pregnancy. In placental mammals, the length of gestation (G) is demonstrated to be related to body weight (W) by the equation $G = a \times W^b$, where a

varies with the type of placenta and *b* is approximately the same (0.17) for all terrestrial animals, but significantly smaller (0.04) for aquatic animals (Kihlstrom, 1972). Prolongation of gestation for most mammals is associated as a rule with reduction in litter size and increase in maturity at birth. In certain mammals, including most of the large ones, natural selection has resulted in increased maturity at birth. The increase in maturity has been associated with, and perhaps may require, prolongation of pregnancy and reduction in the number of young, since large litters are incompatible with long gestations. In these circumstances, an increase in litter size above the norm for which a species is adapted is associated with retardation of fetal growth (McKeown *et al.*, 1976).

(i) Placental size and function

There is a close relationship between placental size and embryo size which has been observed in many mammalian species. The weight of the fetus increases with increasing weight of the placenta in guinea pigs, rabbits, pigs, sheep and mice (Dawes, 1973). Although numerous investigators (Abramovich, 1969; Gellen and Gyori, 1969) have found a positive correlation between fetal and placental size in humans, no differences have been found between normal and large or small for gestational age infants when fetal–placental ratios have been corrected for gestational age (Molteni *et al.*, 1978). This observation emphasizes the importance of placental function rather than placental size. Specifically, uterine and umbilical blood flow, intraplacental metabolism and hormonal control and substrate flux determine fetal growth. Although large babies almost always have large placentas, the converse is not always true. Certain clinical conditions such as high altitude (Kruger and Arias-Stella, 1970), placental haemangiomata and some chromosomal anomalies (Polani, 1974) are characterized by large non-oedematous placentas and growth-retarded fetuses.

Placental blood flow is clearly crucial in regulating the transfer of substrate (e.g. glucose, amino acids, fatty acids; Kretchmer *et al.*, 1989) to both placenta and fetus (Kretchmer *et al.*, 1989). Numerous studies in animals have utilized either ligation or embolization of uterine vessels or surgical reduction of placental area to induce intrauterine growth retardation (Dawes, 1976; Cassady, 1981). Clinical conditions presumably associated with diminished placental blood flow, such as maternal toxaemia, severe diabetes and long-standing hypertension, also result in an increased incidence of small for gestational age babies (Gewolb and Warshaw, 1983).

Placental metabolism is an important variable in determining fetal growth, since it accounts for about half of the apparent utilization of glucose delivered to the placental circulation (Morriss, 1981). Placental production of various polypeptide hormones (human chorionic gonadotrophin, placental adenyl cyclase, human placental lactogen) and steroids (oestrogen and progestins) may be involved in the regulation of substrate flow to the fetus (Chatterjee and Munro, 1977; Mulay *et al.*, 1980).

The growth of the fetus may be adversely affected by one or more conditions leading to placental restriction:

1. reduction in placental size due to abnormal differentiation or limitation of maternal nutrition;
2. diminished uterine blood flow due to various pathological conditions;
3. abnormal development of placental membranes which adversely affects membrane transport properties; and
4. disorders in fetal placental circulation.

6.5 PRENATAL MORTALITY

Spontaneous prenatal mortality is a widespread phenomenon, which occurs in all

species and strains of otherwise normal and fertile domestic and laboratory animals as well as human and non-human primates. Termination of pregnancy may occur at several stages: (a) between fertilization and implantation; (b) during the time of implantation; or (c) after implantation at various stages of embryo or fetal development.

6.5.1 HUMAN AND NON-HUMAN PRIMATES

(a) Incidence

Human fecundability, i.e. the probability of producing a full-term infant per menstrual cycle during which intercourse occurs, is very low when compared with the fertility of domestic animals. Demographic studies have shown that fecundability ranges from 21 to 28% between the age of 20 and 30 years (R.V. Short, 1979). Prenatal mortality at different stages in gestation contributes markedly to this low fertility rate. The estimated frequency of losses after clinical recognition of pregnancy is 10–25%, depending on the epidemiological approach. It has been estimated from the results of studies using different methodological approaches (Hertig *et al.*, 1959; French and Bierman, 1962; Hertig, 1967; Leridon, 1977; J.F. Miller *et al.*, 1980; Rolfe, 1982; Shiota *et al.*, 1987) that a much greater rate of loss of fertilized ova occurs preclinically, i.e. before or during the implantation period (Kline and Stein, 1985). Figure 6.11 graphically illustrates the probability of spontaneous abortion during human pregnancy based on this analysis of the literature.

Prenatal mortality occurs throughout gestation in non-human primates, but the level is particularly high during the early embryonic stages when pregnancy confirmation may be uncertain and/or unreliable. Assessment of the magnitude of early embryonic mortality is additionally complicated by the occurrence of 'placental bleeding', which is frequently seen in macaques during the second and third weeks of normal pregnancy (Hendrickx and Binkerd, 1980). Information on embryonic death during the peri-implantation period has been provided by morphological examination of normal and abnormal embryos in the rhesus monkey (Heuser and Streeter, 1941), baboon (Hendrickx and Binkerd, 1980) and chimpanzee (Heuser, 1940). These studies indicate pre-implantation losses of 26.3% and 25.0% for rhesus monkeys and baboons respectively. Histological features of abnormal embryos at this stage are similar to those described for human embryos and include cell and nuclear fragmentation, organellar aggregation and irregularity in blastomere size and shape (Enders *et al.*, 1982). Corresponding values during the post-implantation period (approximately gestational days 10–22) have been estimated at 14.3% in baboons, 28% in rhesus monkeys and 50% in chimpanzees. Characteristics of embryos lost at this stage, i.e. embryonic degeneration, retardation and asymmetry of the embryonic disc, and abnormalities of the extraembryonic membranes, also parallel observations in defective human embryos.

During the subsequent period of organogenesis (approximately days 20–50), the incidence of embryonic loss is significantly lower for several non-human primates than in the peri-implantation period. Microscopic examination of embryos of five Old World species indicate embryonic mortality rates of 2.4–18.2% during organogenesis (Hendrickx and Binkerd, 1980). The increased frequency of prenatal loss during the early stages of pregnancy has also been well demonstrated in a marmoset breeding colony (Heger *et al.*, 1988). Following a 30% implantation failure rate (including missed fertilization and pre-implantation losses), 28% of implantations failed prior to organogenesis. Lower loss rates occurred during organogenesis (20%) and the fetal period (4%) in this study.

Reported figures for the incidence of stillbirths among 11 species of indoor-housed primate colonies, including the green mon-

key, mangabey, baboon, langur and five species of macaques, range from 5.9% to 20% (Hendrickx and Binkerd, 1980). These rates are markedly higher than the 2.7% figure reported for free-ranging rhesus monkeys (Koford, 1965) and may be attributable to the artificial housing conditions.

(b) Aetiology

The primary factors which have been implicated in prenatal mortality in human and non-human primates are abnormalities of embryonic development, adverse maternal factors and external embryolethal influences (see Rushton, 1985, and Kochenour, 1987, for reviews).

Genetic factors

There is considerable information on the large contribution of an abnormal fetal karyotype to spontaneous abortions in humans (Carr, 1967; J. Boué *et al.* 1975; A. Boué *et al.* 1985; Jacobs, 1982; Warburton, 1987). In most, but not all studies, the proportion of chromosomal anomalies tends to be low (14–26%) in abortions before 8 weeks, highest (40–65%) in losses at 8 – 15 weeks and

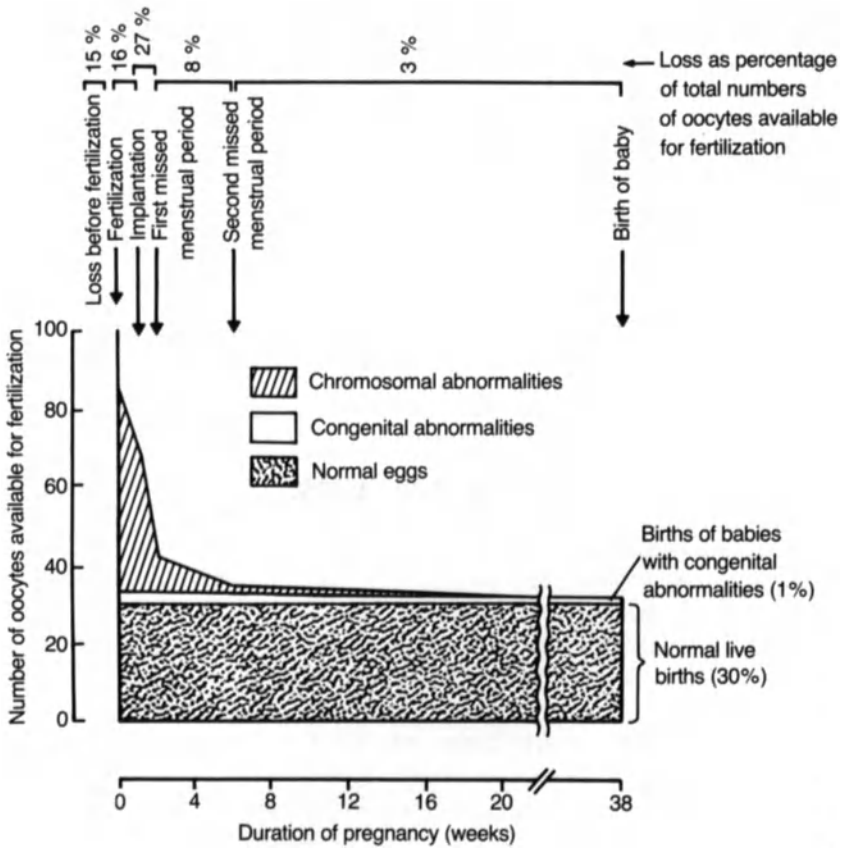

Figure 6.11 The probability of spontaneous abortion during human pregnancy based on the current literature. From these studies it has been estimated that 75% of all fertilizations will result in embryonic or fetal death. The largest proportion of losses occurs in early pregnancy, i.e. 30% prior to implantation, 18% after implantation and the remaining 27% after day 14 of gestation. (Reproduced with permission from Begley *et al.*, 1980.)

decreasing to about 5–10% in abortions after the 20th week of gestation (Leridon, 1977; Warburton *et al.*, 1980). It should be noted that the rate of chromosome anomaly during early pregnancy may be artificially low because of the difficulty in documenting these defects in very early spontaneous abortions (i.e. at less than 4 weeks' gestation). Among chromosomally abnormal spontaneous abortions, approximately one-half are trisomic for one or more chromosomes, 15% are triploid and 20% are monosomy X. The remainder consist of a variety of post-fertilization errors (tetraploidy, hypertetraploidy, hypertriploidy, mosaicism), structural anomalies and double anomalies (Warburton *et al.*, 1980). Both the prevalence and types of chromosomal anomalies observed among recognized spontaneous abortions are considerably greater than those observed among stillbirths and livebirths. This reflects the fact that the majority of chromosomal anomalies (e.g. trisomies, triploidies, monosomy X) are incompatible, or rarely compatible, with survival to birth (Kline and Stein, 1987; J.L. Simpson, 1990). Hormonal imbalance in women, intrafollicular over maturity of ova and the ageing of gamete in the female reproductive tract before gamete fusion have been cited as the most plausible reasons for the high frequency of spontaneous chromosomal aberrations (Dyban and Baranov, 1987). Although chromosomal abnormalities remain the most common single finding associated with abortion, a simple cause-and-effect relationship has not been established between karyotype and abortion in humans (Rushton, 1987). Insufficient data are available to confirm a similarly high contribution of genetic abnormalities to the incidence of abortion in non-human primates.

Maternal factors

Age, parity The association of spontaneous abortion with maternal age in humans has been attributed to two independent causes:

1. the association of trisomic conceptions with age; and
2. the risk of abortion of euploid conceptions with age.

The risk of trisomy at conception, as observed in abortions, rises with maternal age. Some trisomies (e.g. 7, 10, 17, 18, 20–22) contribute more to the increasing risk with age than others (e.g. 2, 8, 9, 16) (Stein *et al.*, 1980). Several studies have shown that the risk of abortion, particularly first abortions (Warburton and Fraser, 1964), rises with increasing maternal age (Naylor, 1974; Selvin and Garfinkel, 1976; Harlap *et al.*, 1980), paternal age (Selvin and Garfinkel, 1976) and parity (Warburton and Fraser, 1964; Naylor, 1974; Harlap *et al.*, 1980). Possible explanations for a non-genetic maternal age effect are cumulative exposure to toxins, greater opportunity to acquire chronic infection, diminished luteal response and poorly vascularized endometrium (J.L. Simpson, 1990). There is little evidence that the increasing difficulty in maintaining pregnancy in older women is due to primary changes in the uterus (Talbert, 1968). Age and parity have also been cited as possible maternal causes of reproductive failure in macaques (see Small, 1982).

Nutrition Although the relationship between nutritional deprivation and reproductive outcome in humans has not been clearly established, it appears that significant restriction of maternal nutrition affects fetal growth, birth weight and post-natal mortality, rather than intrauterine mortality (Kohrs *et al.*, 1976; Susser and Stein, 1980). Studies carried out in rhesus monkeys indicate that normal prenatal growth is maintained on a protein-deficient diet owing to the ability of the gravid primate uterus to adapt to such nutritional restriction (Riopelle, 1985). However, severe protein deprivation during pregnancy has been found to result in maternal as well as fetal mortality in these species (Kohrs *et al.*, 1976).

Health Several medical illnesses have been implicated in causing habitual abortions in women. It has been speculated that the higher rate of fetal wastage associated with systemic lupus erythematosus (Fraga *et al.*, 1973) may be due to immune complex deposition on trophoblastic basement membrane (Grennan *et al.*, 1978) and fetal cardiac anomalies (Chameides *et al.*, 1977). The abnormally high rates of fetal loss (>50%) in women with congenital cardiac disease may be attributable to deficits in the supply of well-oxygenated blood to the developing fetus (McAnulty *et al.*, 1982). Severe maternal illnesses which have also been associated with increased prenatal mortality are Wilson's disease, phenylketonuria, haematological disorders (e.g. haemoglobinopathies, aplastic anaemia), poorly controlled type 1 diabetes mellitus and renal disease with associated hypertension (Felding, 1968; J.L. Simpson, 1990).

Studies have been undertaken in rhesus monkeys to examine the effects of experimentally induced diabetes mellitus on fetal metabolism and growth (Mintz *et al.*, 1972). Administration of the pancreatic beta-cell cytotoxin, streptozotocin, before conception and during the first trimester of pregnancy is associated with a 27% mortality rate during the second and third trimesters. The drug effects included fetal hyperinsulinaemia, enhanced pancreatic islet cell responsiveness, enlarged placentas and polyhydramnios.

Infections Abortion, intrauterine growth retardation, congenital anomalies or severe disease of the newborn may be a result of certain maternal infections. Several reviews of this topic provide information on the major clinical features as well as the fetal risk of infections that have been implicated in obstetric pathology (Sever, 1980; Remington and Klein, 1983; Persaud *et al.*, 1985; Benirschke and Robb, 1987). Intrauterine infection as an aetiological agent for recurrent pregnancy wastage remains a highly controversial clinical question because of the difficulty in separating the adverse effect of an infection on the developing fetus from its effect on the mother (Rushton, 1985).

The following micro-organisms and viruses have been associated with early spontaneous abortion: congenital rubella and rubeola viruses, *Treponema pallidum* (syphilis) (Sever, 1980), smallpox virus (Lynch, 1932), vaccinia (M.M. Levine *et al.*, 1974), *Salmonella typhosa* (Hicks and French, 1905), *Vibrio fetus* (Eden, 1966), *Plasmodium* (Preston, 1942), *Chlamydia* (Schachter, 1967), cytomegalovirus (Kriel *et al.*, 1970; Sever, 1980), *Candida* (Ho and Aterman, 1970), *Brucella* (Sarram *et al.*, 1974), herpesvirus (Nahmias *et al.*, 1971; Sever, 1980), *Toxoplasma* (Zighelboim *et al.*, 1968; Kimball *et al.*, 1971; Sever, 1980) and *Mycoplasma* (Kundsin *et al.*, 1967).

Both spontaneous and experimentally induced maternal infections have been shown to be important factors in reproductive failure in some species of non-human primates. The following infections have been implicated as causative agents in the observed high level of abortions or stillbirths in non-human primates: Chagas' disease (*Trypanosoma cruzi*-like) in marmosets (Lushbaugh *et al.*, 1969); T-strain mycoplasmas in talapoins (*Miopethecas talapoin*) and patas monkeys (*Erythrocebus patas*) (Kundsin *et al.*, 1975); measles virus in rhesus monkeys (Hertig *et al.*, 1971); rubella virus in crab-eating monkeys and baboons and mumps virus in rhesus monkeys (Hendrickx, 1966; Delahunt and Rieser, 1967; London *et al.*, 1971).

Stress Although several clinical studies have shown an association between psychological disturbances and habitual abortion, evidence demonstrating a cause-and-effect relationship is lacking (Weil and Tupper, 1960; Grimm, 1962). Among the psychological stresses related to an adverse pregnancy outcome are bereavement (Kaij *et al.*, 1969;

Mandell and Wolfe, 1974) and neurotic illnesses (Mandelbrote and Monro, 1964). Although difficult to substantiate, physical stress (e.g. heavy lifting and physical effort) has also been implicated as a risk factor in spontaneous abortions (Goulet and Theriault, 1987). The physiological mechanism remains unclear; however, fetal hyperthermia and changes in circulating maternal hormone levels leading to uterine contractility have been proposed.

Observations in rhesus monkey colonies indicate that maternal psychological factors may be as important as maternal physiological conditions during pregnancy for fetal viability. The high level of abortion (50–70%) in pregnant animals captured in a native environment and shipped to the USA for experimental purposes may be partially attributable to the high degree of stress associated with handling techniques (Myers, 1972). Experiments carried out in rhesus monkeys to study the effect of maternal stress on pregnancy indicate that excitability and pain associated with labour and delivery may have deleterious effects on the fetus. Brief episodes of experimentally induced stress in near-term rhesus monkeys cause fetal deterioration in the form of fetal bradycardia and decreased arterial oxygenation (Morishima *et al.*, 1978) or fetal asphyxia and concomitant disturbances in acid–base balance (Myers, 1975) as a result of impaired uteroplacental circulation. Boot *et al.* (1985) have also demonstrated that housing conditions (i.e. cage size and density) can adversely affect pregnancy outcome which may be related to stress in cynomolgus monkeys.

Immunological abnormalities Disturbances in the maternal immune system which normally allows the fetus to survive within the uterus without being rejected may play a role in early abortion. Evidence suggests that immunological aberrations, rather than genetic factors, may have a greater contribution to repeated pregnancy failure (J.R. Scott *et al.*, 1987). Although the mechanisms are unclear, the increased rate of fetal death associated with autoimmune disease may be related to uteroplacental vascular lesions (Branch, 1987). Potential alloimmune mechanisms in pregnancy wastage include:

1. absence of serum blocking antibodies which are normally produced by the maternal immune system as an initial response to the implantation of the blastocyst (Rocklin *et al.*, 1976);
2. homozygosity of histocompatibility locus antigens (HLA) between partners which may result in a failure of the maternal immune system to produce blocking antibodies (Komlos *et al.*, 1977; Beer *et al.*, 1981);
3. reduction in early pregnancy factor (EPF), which normally suppresses maternal lymphocyte activity and immune response to the fetal allograft during early pregnancy (Smart *et al.*, 1981);
4. ABO blood group incompatibility between the father and mother (Lauritsen *et al.*, 1976);
5. the existence of antisperm antibodies in the mother which may cross-react with cleaving embryos (W.R. Jones, 1976);
6. the aberrant expression of transplantation antigens on the placental surface (T.J. Gill, 1990); and
7. the presence of anti-phospholipid antibodies in the mother (H.L. Brown, 1991).

Endocrine

It is uncertain whether the majority of endocrine disturbances reported in association with spontaneous abortion reflect the failure of the pregnancy rather than the cause of that failure (Rushton, 1985). Although still controversial, it has been suggested that progesterone deficiency may play a role in some cases of infertility. The incidence of luteal phase deficiency (LPD) has been reported to range from 3.7% (G.S. Jones and Pourmand, 1962)

to 10.7% (Gillam, 1955) in infertile patients and to be 35% in habitual aborters (G.E.S. Jones and Delfs, 1951).

Uterine factors

A variety of congenital anomalies of the uterus have been implicated as causative factors in reproductive wastage in humans. These Mullerian fusion anomalies include unicornuate, didelphic, septate and bicornuate uteri (Rock and Jones, 1977; Rock and Zacur, 1983). An increased rate of early spontaneous abortion has also been associated with congenital alterations in uterine blood supply, which may result in poor placental growth and development (Burchell *et al.*, 1978).

Habitual abortion has been noted in 14% (238 of 1973) of patients with uterine adhesions reviewed in the literature (Schenker and Margalioth, 1982). Early pregnancy loss may be due to the decreased dimension of the uterine cavity and absence of sufficient endometrium for fetal growth or defective vascularization of scanty endometrium (Polishuk *et al.*, 1977; Rock and Zacur, 1983). Uterine leiomyomas responsible for repetitive pregnancy loss have been estimated at approximately 18% (Robins, 1972). Alterations in endometrial stroma or vasculature leading to abnormal implantation or decreased blood supply to the developing placenta and fetus may be causes of fetal wastage associated with fibromyomas (Farrer-Brown *et al.*, 1971). Although it is difficult to document dystocia in non-human primates due to the high incidence of night births, contributing causes may include cephalopelvic disproportion, positional abnormalities, uterine malformation and inertia, uncontrolled haemorrhage and toxaemia (Hendrickx and Binkerd, 1980). Adverse pregnancy outcome (i.e. stillbirth) has also been associated with breech rather than cephalic presentation in term pregnancies of cynomolgus (Cho *et al.*, 1985) and pig-tailed (Goodlin and Sackett, 1983) macaques.

Various types of placental insufficiency have been associated with high rates of fetal mortality in the human population as well as in some non-human primate species. These include infections of the placenta (placentitis), impaired placental circulation (infarctions and abruptio placenta) and abnormal placental location (placenta praevia).

Placental infections in humans are often responsible for fetal death and sometimes associated with maternal morbidity and mortality. Infections reach the placenta by ascent from the lower genital tract, by contiguity with endomyometrial lesions, transtubally and via the maternal circulation. While bacteria are the most common pathogens to the placenta, fungal, viral and parasitic infections also contribute to this type of placental pathology (Benirschke and Driscoll, 1967; Rushton, 1985).

In a histological study of stillborn fetuses from a variety of non-human primate species, necropsy examination confirmed placentitis in seven of eight cases as the primary cause of fetal demise. The most commonly isolated organisms which were responsible for ascending genital infections leading to placentitis and subsequent fetal anoxia were group D streptococci and β-haemolytic, coagulase-positive *Staphylococcus aureus*. Infection of the placenta was accompanied by infiltration of inflammatory cells, oedema, necrosis and haemorrhage which interfered with fetal oxygenation (Andrews, 1974). Gram-positive cocci, especially α-haemolytic *Streptococcus viridans*, were implicated in 11 of 17 cases of abortions and stillbirths in rhesus monkeys (Swindle *et al.*, 1982). Acute placentitis and fetal bronchopneumonia were the most consistent histopathological findings in these cases.

Abruptio placenta, a premature separation of the placenta from the endometrial surface, is a serious obstetric complication which occurs in 1–2% of human pregnancies. This condition accounts for 1% of maternal mortality and 15% of all perinatal mortalities. The

mortality rate for infants of abruption ranges from 30–50% (Berstein, 1981). The reported incidence of abruptio placenta in 1200 rhesus monkey pregnancies was 0.6% and was also associated with a high degree of fetal death (Myers, 1972). The recent use of ultrasonography in primate breeding colonies has considerably reduced the mortality associated with this condition. Of nine concealed abruptions detected over a 4-year period (1% incidence), no maternal deaths occurred as all animals were scheduled for hysterotomies upon ultrasonic detection of retroplacental or subchorionic haemorrhage (Tarantal and Hendrickx, 1988b).

Placenta praevia, which is characterized by partial or complete placental localization over the cervical outlet, may be associated with a high risk of bleeding and an increased hazard of ascending infection during pregnancy. The clinical frequency of this condition averages approximately one in 250 births (0.4%) (Green, 1984) and the risk of recurrent placenta praevia is as high as 8% (Kelly and Iffy, 1981). Perinatal survival associated with this problem is critically dependent on the gestational age at delivery. Seventy-three per cent of all perinatal deaths are attributable to praevia deliveries prior to 32 weeks' gestation, which occur in 20% of affected women (Cotton *et al.*, 1980). This condition also leads to an increased level of fetal loss in rhesus monkeys, in which the reported incidence has been estimated at 0.5% among 1200 cases (Myers, 1972; Hendrickx and Binkerd, 1980).

Environmental factors

Intrauterine death is the most common result of a teratogenic insult early in embryonic development when the undifferentiated embryonic cells are more refractory to teratogenesis. After the initiation of organogenesis, malformations of specific organs or systems, in addition to variable degrees of embryolethality and growth retardation, are the primary manifestations of hazardous environmental factors. Interference with development during the subsequent periods of histogenesis and functional maturation would be expected to result primarily in growth retardation and functional disturbances.

The relationship between intrauterine death and malformations following exposure to a teratogenic insult has not been clearly established. Some authorities view death and abnormal development as simply different degrees of reaction to the same stimuli, with the rate of mortality and the rate and severity of malformations increasing in roughly parallel fashion as dosage is increased (Beck and Lloyd, 1963; J.G. Wilson, 1959). Other investigators (Gebhardt, 1970; Tuchmann-Duplessis, 1970) cite a distinction between embryolethality and teratogenicity as unrelated responses. This issue is a critical one in the interpretation of teratological evaluations and may reflect interspecies differences in sensitivity to environmental toxins. These could be attributed to differences in genetic make-up as well as species-specific reproductive features, particularly length of gestation (Schardein, 1976).

It has been suggested that the characteristics of the agent itself may determine the pattern of response, i.e. environmental teratogens can be divided into those that cause non-specific fetal death and those that cause malformations which result in fetal death. It may be possible that one agent, under different circumstances (e.g. dose and time of exposure), may exhibit both these properties (Kalter, 1980).

In a recent tabulation of potential human developmental toxicants, the primary manifestation of adverse outcome was fetal/neonatal death. Among the agents associated with pregnancy loss were cigarette smoking and alcohol in addition to drugs (e.g. retinoic acid) and environmental chemicals (e.g. lead, organic mercury) (Schardein and Keller, 1989).

Several agents have been shown to cause embryolethality in the absence of malforma-

tions as the primary manifestation of developmental toxicity in the rhesus, cynomolgus and pig-tailed macaques. These include a variety of natural and synthetic sex steroids in addition to antineoplastic agents (methotrexate, 5-fluorouracil), therapeutic drugs (norfloxacin, diphenylhydantoin, acetazolamide) and alcohol (Binkerd *et al.*, 1988). A number of additional agents (e.g. valproic acid, triamcinolone acetonide, retinoic acid) are associated with malformations and/or intrauterine growth retardation in addition to prenatal mortality in a variety of primate species (Hendrickx and Binkerd, 1990).

6.5.2 DOMESTIC AND LABORATORY SPECIES

(a) Incidence

The methods used to demonstrate the existence and extent of prenatal death are variable depending on the species and the period of gestation in question. In cattle, estimates of early pregnancy loss are made by calculation of the fertilization rate and the number of fertilized ova that fail to continue development following slaughter at different intervals after mating (Laing, 1949). Although less reliable, calculation of the return to oestrus interval following mating has also been used as a reflection of the extent of embryonic death (D.L. Stewart, 1952). Information on prenatal death occurring during the late embryonic and fetal periods has been obtained by comparison of the results of pregnancy diagnosis by rectal palpation at 30–40 days with subsequent pregnancy diagnosis or with calving rate (Hanly, 1961).

In the pig, since total loss of pregnancy is apparently uncommon, the effect of prenatal death takes the form of a reduction in litter size (R.F. Wilson *et al.*, 1949) and information is obtained by comparison of the number of corpora lutea with the number of surviving embryos or with litter size (King and Young, 1957). In sheep, the methods based on slaughter and on delay in oestrous return interval have been used (Hanly, 1961).

In most domestic animals the highest rate of prenatal mortality occurs during the first 4 weeks of gestation which coincides with the period of rapid growth and differentiation of the embryo and the extraembryonic membranes. In cattle, which are usually monotocous, fertilization failure rates of 15% have been reported (H. Boyd *et al.*, 1969) and estimates of embryonic mortality range from 15–34% during the first 6 weeks of gestation (Robinson, 1957; R.V. Short, 1979). The subsequent losses before term are low and range from 3–5% (Ulberg, 1962). Egg transfer studies indicate that asynchrony between the endocrine state of the uterus and the stage of development of the fertilized egg results in embryonic death (Betteridge and Mitchell, 1974). Proximity to another embryo also reduces the chances of survival, as shown by a doubling of the chance of twins when one egg is shed from each ovary as compared with both from one (Gordon *et al.*, 1962) or when one is transferred to each uterine horn (Rowson *et al.*, 1971). Infectious agents have been reported to account for approximately 23% of abortions and stillbirths in cattle in the USA (Hubbert *et al.*, 1973).

In sheep, which are usually polytocous, there is a high fertilization rate of 90–100%, but 20–30% of these fertilized ova fail to develop to full-term lambs, with most of the losses occurring before day 18 of pregnancy (Quinlivan *et al.*, 1966; Edey, 1976). Infectious agents rank high as the primary cause of early embryonic loss in this species (W.J. Scott, 1978). Additional contributing factors include high ambient temperature, nutritional extremes and genetic abnormalities. The stillbirth rate in sheep has been estimated at 2–13% (Terrill, 1962).

The pig is another polytocous species with a high fertilization rate (95–100%) which experiences the greatest level of embryo loss, 25–50%, during the first 25 days of pregnancy (Scofield, 1975; Selby, 1978). While most

abortions result from infectious diseases, other causes include systemic diseases, high ambient temperature, physical injuries and chemicals or drugs with abortifacient actions (e.g. oestrogens). The estimated 6% stillbirth rate in this species has been attributed to maternal dystocia, nutrition, age and health as well as hereditary factors (Day, 1962).

The average abortion rate is lower in horses, i.e. approximately 10%. Some reports indicate that the mares are endocrinologically most susceptible to abortions during the fifth month owing to hormonal deficiencies that can result when the placenta takes over progesterone synthesis from the ovary. Decreased progesterone production during the 10th gestational month also accounts for the higher frequency of fetal death during this period (Nishikawa and Hafez, 1962).

Prenatal mortality in laboratory species also occurs to the greatest degree during early pregnancy. The estimation of pre-implantation embryo loss is most commonly based on a comparison of the number of corpora lutea with the number of implantation sites. However, the accuracy of this method is limited since it makes no allowance for failed fertilization (J.G. Wilson, 1978). Further, the number of corpora lutea may not correspond to the number of ova released because of the occurrence of polyovular follicles and anovular corpora lutea that have been reported for some species (Kent, 1962; Horowitz, 1967). Post-implantation loss is more accurately assessed by comparing the number of live fetuses at term with the number of implantation sites in those species with short gestation periods that have recognizable signs of endometrial decidualization at term, e.g. rats and other rodents (J.G. Wilson, 1978).

In the rabbit, there is an estimated 10–11% loss prior to implantation and 15–18% after implantation (Adams, 1960; Palmer, 1978). In addition to such extraneous causes as disease, poor nutrition and treatment with embryotoxic agents, variability in prenatal mortality is largely genetically determined. While asynchrony between embryo development and endometrial stage accounts for a large part of the pre-implantation mortality (Noyes and Dickman, 1960), deficiency in placental development or inadequacy of endometrial vascular supply contributes to post-implantation losses (Hafez and Tsutsumi, 1966).

In laboratory rats pre-implantation loss ranges from 6–8% and post-implantation loss between 5% and 7% (J.G. Wilson, 1978). The corresponding overall prenatal loss in wild rats is 25% (Perry, 1945). In ferrets the pre- and post-implantation loss has been estimated at 15% (Marston and Kelly, 1969). Only post-implantation losses have been reported in guinea pigs (5–7%) (Hoar, 1978; Shenefelt, 1978) and golden hamsters (7% average) (Shenefelt, 1978). Reported values for prenatal loss in wild and laboratory mice are 31–36% and 24–36% respectively (Berry, 1981). Very little information is available on the level of prenatal mortality in dogs and cats. However, a 5% rate of resorptions on day 60 (Earl, 1978) and a 7% rate of stillbirths (Bowden *et al.*, 1963) has been reported for dogs.

(b) Aetiology

Prenatal mortality in domestic and laboratory animals can be due to genetic factors, maternal factors or adverse environmental conditions (Wilmut *et al.*, 1986).

Genetic factors

The frequency of embryonic loss in domestic animals is partially determined by the genetic make-up of the sire (Perry, 1960) and the dam (L.N. Baker *et al.*, 1958) as well as the breeding system. In swine (Squiers *et al.*, 1952), cows (Hawk *et al.*, 1955; Mares *et al.*, 1958) and rabbits (Palmer, 1978) inbreeding increases and cross-breeding reduces the

embryonic mortality rate. Breed differences in sheep affect the level of prenatal death, i.e. some breeds are more susceptible to embryonic loss than others (Foote *et al.*, 1959).

The effect of semen characteristics on embryonic mortality is pronounced, especially where artificial insemination is practised. In the pig (Dziuk and Henshaw, 1958) and the sheep (Dutt and Simpson, 1957), the quality of semen used for artificial insemination by donor (AID) has a direct effect on embryo survival. Knudsen (1956) has correlated abnormal chromosome make-up in bull sperm with increased embryonic death, presumably as a result of an unbalanced genetic constitution of the embryo.

There appears to be great variability between species, breeds, strains and families in the incidence and types of aberration causing embryonic mortality (Gustavsson, 1980). Spontaneous chromosome aberrations have been estimated to occur in embryos in frequencies of 1–8% in the rabbit (Hofsaess and Meachan, 1971; Martin and Shaver, 1972; Fechheimer and Beatty, 1974) and 12.5% in the pig (McFeely, 1967). The predominant aberrant karyotypes are triploids, and it has been shown that at least in species such as the pregnant rabbit they survive until mid-term (Bomsel-Helmreich, 1965). Haploidy, pure heteroploidy and pure polyploidy are rarely observed in domestic animals born at full-term since most affected fetuses probably die early in gestation (Gustavsson, 1980). Embryonic studies have demonstrated the occurrence of polyploidy in pig blastocysts (Moon *et al.*, 1975), tetraploidy in cattle embryos (McFeely and Rajakoski, 1968) and trisomy in cleavage-stage sheep embryos (Long and Williams, 1978). Unbalanced karyotypes and autosomal trisomy in particular, are much less common in laboratory rats and mice than in humans. The most common chromosome aberration in these rodents is triploidy, which is inevitably lethal by mid-gestation (Dyban and Baranov, 1987). In both species, the presence of major histocompatablity (MHC)-linked recessive lethal genes is associated with *in utero* death at different stages of development.

Maternal factors

Age It has been well established that the mean litter size at birth decreases with advancing maternal age in many polytocous species, including the rat (Ingram *et al.*, 1958), mouse (E.C. Jones and Krohn, 1961), pig (Perry, 1954) and hamster (Soderwall *et al.*, 1960). This decrease is well correlated with increasing levels of prenatal mortality (Hollander and Strong, 1950; Finn, 1962; Connors *et al.*, 1972; Kalter, 1975) rather than with exhaustion of oocytes in many mammalian species (E.C. Jones and Krohn, 1961; Talbert, 1978). In contrast, lower levels of embryonic mortality associated with increasing age have been reported in cattle (Erb and Holtz, 1958). The relative importance of ageing of ova and the uterus as factors in the decline of reproductive capacity appears to vary considerably from one species to another. Data presently available indicate that this age change in the mouse is almost exclusively the result of a decline in the ability of the uterus to support pregnancy (Talbert and Krohn, 1966; Gosden, 1975), whereas in the hamster (Blaha, 1964), and to a lesser degree in the rabbit (Adams, 1970; Maurer and Foote, 1971), degenerative changes in the ovum and in the uterus both promote reproductive decline.

Nutrition There is considerable evidence that specific nutrients, level of feeding and/or body condition may influence conception rates and embryonic survival in most species of domestic and laboratory mammals (Rattray, 1977). In swine (Self *et al.*, 1955; Haines *et al.*, 1959) and sheep (El-Sheikh *et al.*, 1955; Foote *et al.*, 1959), high caloric intake or continuous unlimited feeding increases ovulation rate as well as embryonic mortality up

to the time of implantation. The effect of caloric intake on prenatal death in cattle is controversial (Hafez, 1967; Rattray, 1977).

Studies in rats (Nelson and Evans, 1953; Berg, 1967) have verified the requirement for an adequate protein source in the earliest stages of placental and fetal development. A high level of early resorption in this species results when animals receive a severely protein-restricted diet during early pregnancy. Experimental studies in mice have shown that decidual hemorrhage and necrosis accompanied by embryonic mortality can be induced by short-term fasting (McClure, 1959, 1961).

Infections Infectious abortions account for a significant portion of the reported reproductive failure in several domestic animals (Hubbert *et al.*, 1973; W.J. Scott, 1978; Holter and Andrews, 1979). Table 6.8 lists the major infectious diseases and organisms which have been identified in the aetiology of prenatal mortality in cattle, sheep, swine, goats, dogs, cats and horses.

Overcrowding in utero

Transuterine migration of embryos is of special physiological significance in certain polytocous species for equal distribution of conceptuses between the two uterine horns. In swine there is a high incidence of embryonic mortality in the absence of migration (Smidt, 1962). Embryo transfer experiments in cattle have demonstrated a greater degree of embryonic loss in cows which had received two transferred embryos in a single uterine horn, presumably because of overcrowding and intrauterine competition for nutrients (Rowson *et al.*, 1971). In rabbits (Hafez, 1964) and guinea pigs (Eckstein *et al.*, 1955), as the number of implantations rise, the vascular supply to each site is reduced, which restricts placental development and causes increased levels of *in utero* death.

Environmental factors

Surveys of veterinary diagnostic laboratory data in the USA have verified the following environmental toxins as causative factors in non-infectious abortions in cattle and swine: mycotoxicoses, nitrate–nitrite compounds, carbon monoxide, selenium deficiency, lead poisoning, chlorinated naphthalene, locoweed, pine needle and perennial broomwood (Stuart and Oehme, 1982).

Increased embryonic mortality occurs in a number of species following exposure of the female to elevated ambient temperature (Ulberg, 1958; Dutt, 1960). High summer temperature has been shown to have a detrimental effect on spermatogenic activity in rams, which causes decreased fertilization as well as increased embryonic mortality rates (Dutt and Simpson, 1957). In the rabbit, transfer of fertilized ova following culture at 40°C results in increased levels of post-implantation mortality (Alliston *et al.*, 1965). Reciprocal ova transplantation experiments in this species have shown that elevated temperatures have adverse effects on the maternal tissues rather than on the embryo (Shah, 1956). Experimentally induced thermal stress in cattle (Ragsdale *et al.*, 1948), pigs (Tompkins *et al.*, 1967) and rats (Hsu, 1948) results in a high proportion of embryonic mortality.

Studies carried out in laboratory species to develop animal models for potential human teratogens have demonstrated a strong relationship between fetal wastage and exposure to potential human teratogens for several substances. Several agents are known, when given at sensitive times in gestation, to produce mainly or only embryolethal effects in test species, e.g. chloramphenicol, actinomycin D and other antibacterial agents, most folate antagonists, inhibitors of protein synthesis and many post-coital anti-fertility drugs (Wilson, 1980). Other agents which produce increased levels of prenatal death in addition to malformations include: alcohol

Table 6.8 Infectious diseases affecting reproduction in domestic animals (Reproduced with permission from Osebold, 1977)

Disease	*Species*	*Aetiology*	*Reproductive effect*
Bacterial infections			
Brucellosis	Cattle	*Brucella abortus*	Late abortion, sterility in bulls
	Sheep, goats	*Brucella melitensis*	Abortion
	Swine	*Brucella suis*	Abortion, weak pigs
	Dogs	*Brucella canis*	Abortion
Vibrosis	Cattle	*Campylobacter fetus*, subspecies *fetus*	Infertility, early abortion
	Sheep	*Campylobacter fetus*, subspecies *intestinalis*	Abortion in last trimester
Leptospirosis	Cattle	*Leptospira pomona*	Abortion in last trimester
	Swine	*Leptospira pomona*	Late abortion, weak pigs
	Horses	*Leptospira pomona*	Late abortion
Listeriosis	Cattle, sheep	*Listeria monocytogenes*	Abortion – second half gestation
Epididymitis in rams	Sheep	*Brucella ovis*	Infertility in rams, late abortion
Viral infections			
Equine virus abortion	Horses	Equine herpesvirus I	Abortion in last trimester
Equine viral arteritis	Horses	Equine arteritis virus	Abortion – second half gestation
Infectious bovine rhinotracheitis or pustular vulvovaginitis	Cattle	IBR-IPV virus	Abortion – second half gestation temporary infertility in cows and bulls, and fetal infection
Bovine viral diarrhoea	Cattle	BVD-MD virus	Abortion in first trimester, fetal deformities
Epizootic bovine abortion	Cattle	Virus?	Abortion in last trimester
Enzootic abortion of ewes	Sheep	*Chlamydia psittaci*	Late abortion
Blue tongue	Sheep	Modified live virus vaccine	Fetal deformities
Hog cholera	Swine	Modified live virus vaccine	Fetal deformities
Protozoan infections			
Trichomoniasis	Cattle	*Trichomonas fetus*	Abortion in first trimester, sterility in cows
Toxoplasmosis	Cattle, sheep, swine, dogs, cats	*Toxoplasma gondii*	Abortion, stillbirths, premature births, fetal abnormalities

(Kronick, 1976; G.F. Chernoff, 1977; Randall *et al.*, 1977) and nicotine (Nishimura and Nakai, 1958) in mice; methotrexate (J.G. Wilson, 1978), ionizing radiation (Brent, 1977) and alcohol (Tze and Lee, 1975; Abel and Dintcheff, 1978) in rats; nitrates and nitrites (Sleight and Atallah, 1968), norethynodrel (Foote *et al.*, 1968), thalidomide (Arbab-Zadeh, 1965) and vitamin C (Neuweiler, 1951) in guinea pigs; nitrogen mustard in ferrets (Beck, 1978) and glucocorticoids (Howarth and Hawk, 1968; Fylling *et al.*, 1973) and colchicine (Edey, 1967) in sheep.

6.6 DEVELOPMENT OF THE REPRODUCTIVE TRACT

6.6.1 GENETICS OF SEX DIFFERENTIATION

The development of an animal into a male or a female depends on a switch mechanism which is under environmental (e.g. alligators), chromosomal (e.g. *Drosophila*) or genetic control (e.g. mammals). In the last case, the TDF (testis-determining factor) gene on the Y chromosome is responsible for male development (McLaren, 1990). Since it is likely that many different genes are required for both maleness and femaleness, studying the mode of action of one or more of these genes is essential to our understanding of the genetic controls in mammalian sexual development.

The primary development of the mammalian bipotential gonad toward a male or female identity is genetically determined, while the subsequent development of secondary sexual characters is under endocrine control. In most mammalian species, including humans, the female is homogametic and has two isomorphic sex chromosomes (the X chromosomes), while the male has one X and one Y chromosome. It is the presence of a Y chromosome, not the number of X chromosomes, that distinguishes male from female: abnormal individuals with an XO chromosomal constitution are female while XXY genotypes are phenotypically male. Genetic sex is determined at fertilization when either an X- or Y-bearing sperm unites with the X-bearing ovum.

Largely through cases of abnormal sexual differentiation, we have learned that this XX/XY system is not as simple as it first appears. Several genes on each sex chromosome, as well as genes on the autosomes, are known to influence sexual determination and differentiation. The gene products of these loci have not been identified, and the mechanisms by which they steer developmental processes – e.g. as cell recognition markers (L. Singh *et al.*, 1984) or by setting the timing and rate of such processes (Mittwoch, 1983) – are still not understood, although significant advances are being made (Eicher, 1988; Jost and Magre, 1988).

Furthermore, while the XX/XY system characterizes most mammalian species, there are several exceptions or variations on this theme. In some mongooses (genera *Herpestes*, Fredga, 1972; and *Atilax*, Pathak and Stock, 1976) there are apparently no Y chromosomes, so males are determined by the XO configuration. Y to autosome translocations have been postulated based on cytogenetic evidence (*Aotus*) and the observation of trivalents at meiosis including the X chromosome and two homologous autosomes, one of which presumably carries not only the male-determining part of the Y but also the Y–X pairing region (*Herpestes*) (Austin *et al.*, 1981).

A number of mammalian species have multiple sex chromosome mechanisms for sex determination in which more than one type of X or Y chromosome is present (Austin *et al.*, 1981). The general principle that the presence of one or more Y chromosomes corresponds with male sexual differentiation while their absence is associated with female differentiation also holds in these systems.

(a) The Y chromosome

Gonadal determination

H-Y antigen Individuals from highly inbred strains of mice are typically able to accept tissue grafts from members of the same strain. In 1955, however, Eichwald and Silmser reported that females of certain inbred strains gradually rejected skin grafts from syngeneic males, while male-to-female and within-sex grafts were successful. These results suggested the existence of a minor histocompatibility (H) antigen whose presence on male skin cells is controlled by the Y chromosome (hence the name H-Y). Subsequent investigation revealed that H-Y antigen is present on the surface of all nucleated male cells in many mammalian tissues (D.L. Gasser and Silvers, 1972) and that it shows extreme evolutionary conservation, not only within mammals (Wachtel *et al.*, 1974; Nagai and Ohno, 1977; Selden and Wachtel, 1977) but also between different vertebrate classes where its presence is associated with the heterogametic sex (Wachtel *et al.*, 1975a).

Wachtel *et al.* (1975b) proposed that the H-Y antigen was the product of testis-determining genes normally found on the Y chromosome. A number of *in vitro* studies support this hypothesis (Ohno *et al.*, 1978; Zenzes *et al.*, 1978a,b; Muller and Urban, 1981) and several do not (R.V. Short, 1979; Muller and Urban, 1981; Goldberg, 1988). Nonetheless, an association between H-Y and the development of testicular structure seems clear. Evidence that H-Y also directs functional development of the testis comes from the fact that rat ovarian cells reaggregated in the presence of H-Y developed LH/hCG (luteinizing hormone/human chorionic gonadotrophin) receptors, which are characteristic of newborn testes but not ovaries (Muller *et al.*, 1978b). The reaggregation of ovarian cells into testosterone-producing bodies, however, has not been achieved.

There are two kinds of receptors for H-Y antigen. One is associated with the major histocompatibility complex–β2-microglobulin dimers on the plasma membrane and seems to be unable to bind exogenous H-Y. In mouse and human male somatic cells, however, there is an association between H-Y and this dimer (review in Meck, 1984). The second receptor is gonad specific and is able to bind exogenous H-Y both in males and in females (Muller *et al.*, 1978a). Muller *et al.* (1978a) and Russell *et al.* (1982) have suggested that the former receptor sites bind H-Y exerting the histocompatibility function, whereas the gonad-specific receptors, picking up H-Y in solution, are necessary for testis induction. Thus, primary sexual differentiation of the gonad would depend on both the production and reception of H-Y: the receptor is essential.

The genetics of H-Y antigen has been investigated by measuring H-Y titres in individuals with varying chromosomal constitutions. In all but a few reported cases, H-Y was present whenever there were testes (E. Simpson, 1982; Gore-Langton *et al.*, 1983; Goodfellow, 1986). However, H-Y can also be present in the absence of testicular tissue and therefore is clearly not the only determinant of gonadal sex (McLaren *et al.*, 1984; E. Simpson *et al.*, 1984, 1986; Goldberg, 1988). There is also recent evidence that the H-Y antigen may be more involved in spermatogenesis than sexual differentiation (McLaren *et al.*, 1984; Burgoyne *et al.*, 1986; Burgoyne, 1988).

Before leaving the topic of H-Y antigen, we should briefly consider where on the Y chromosome the testis-determining gene is located. Previous studies of Y structural anomalies suggest that the critical locus is on the short arm of the Y near the centromere (reviewed in Davis, 1981). Promising evidence for a more specific location of the testis determining region has recently appeared. Sinclair *et al.* (1990) have located TDF on 35 kilobases of segment 1A1 of the short arm of

the human Y chromosome and is Y specific for a number of mammals, and has been called SRY (sex-determining region of the Y).

Bkm sequences

Bkm sequences are minor satellite DNA sequences, interspersed with other more complex sequences. In the mouse, Bkm sequences are located on the Y chromosome, where they are especially concentrated at the proximal tip and Sxr locus (L. Singh and Jones, 1982), on two regions of the X chromosome and on the autosomes (Kiel-Metzger and Erickson, 1984), including one locus where deletions can lead to hermaphroditism (Kiel-Metzger *et al.*, 1985).

The discovery that Bkm sequences in humans are virtually absent from the Y, while they are concentrated only on the X and two autosomes (Drumm *et al.*, 1985; Kiel-Metzger *et al.*, 1985), necessitates rethinking about the relationship between Bkm sequences and sex determination (Kiel-Metzger *et al.*, 1985). The higher concentration of Bkm sequences found on some heterogametic sex chromosomes is evidently not required for determination of the heterogametic sex. Either Bkm sequences and sex determination are not related, or there are small but important sex differences in the Bkm sequences on the homogametic sex chromosome or autosomes and these sequences are able to fulfil the sex-determining function.

The Y chromosome and spermatogenesis

In man, small deletions of the euchromatic part of the Y chromosome long arm have been associated with azoospermia and suggest that certain Y loci are needed for normal spermatogenesis. Some possibly contradictory cases in which Y deletions coincided with normal fertility have also been described (Davis, 1981).

Further evidence of Y involvement in spermatogenesis comes from XO Sxr (sex reversed) male mice. These animals are believed to have the testis-determining sequence (normally found on the Y chromosome) transferred to the X chromosome. However, although their germ cells do undergo meiosis, these males are ultimately sterile because the spermatozoa are morphologically abnormal (Lyon *et al.*, 1981). Perhaps the lack of a Y chromosome in the germ cells causes their abnormality. Alternatively, it may result from the X's inability to pair with a homologue during meiosis (McLaren, 1983).

While one Y chromosome is evidently required for normal spermatogenesis, more than one Y disturbs this process. XYY humans and mice have testes, but many tubules show arrested spermatogenesis. Normal spermatogenesis is sometimes possible if the second Y chromosome is lost from the germ cell line (R.V. Short, 1982). A disorderly meiosis may again be the cause of the problem.

The Y chromosome and oogenesis

It is unlikely that the Y chromosome prevents oogenesis (McLaren, 1983). In XX/XY mosaic mice, XY germ cells can develop as oocytes, though this very rarely occurs (C.E. Ford *et al.*, 1975; Evans *et al.*, 1977). XX oocytes predominate in such chimaeras, but this is probably because of the advantage of having a second X (see below).

(b) The X chromosome

Gonadal determination

The X chromosome appears to have a repressing effect on the action of the Y chromosome, hence inhibiting organization of the testis. Evidence comes from the study of XY females whose sex reversal is familial and X-linked. The best known of such cases is the Scandinavian wood lemming, *Myopus schisticolor*, which received attention because some females produce only female offspring.

In this species, as well as in the Alaskan lemming *Dicrostonyx torquatus* (Gileva and Chebotac, 1979), some fully fertile females have an XY genotype, though they produce only XX oogonia (Fredga *et al.*, 1976). These females receive normal Y chromosomes from their fathers, so an X-linked locus (X*) must lead to sex reversal. The X* chromosome is slightly shorter than a wild-type X, owing to deletion and structural rearrangements in the short arm (E.W. Herbst *et al.*, 1978). Although the exact mechanism of sex reversal is unclear, females carrying X* transmit it to their XY offspring who consequently develop as females.

Further evidence that the X* chromosome has lost genes necessary for male sexual development comes from X*XY sterile males, which occur occasionally as a result of failure of the double non-disjunction which typifies X*Y meiosis. Schempp *et al.* (1985) determined that when the wild-type X chromosome was inactivated X*XY individuals were fertile females. When the mutant X* was inactivated, X*XYs were males which were sterile owing to the presence of two X's.

Cases of familial XY gonadal dysgenesis (X-linked recessive) are also known in humans (Sternberg *et al.*, 1968; German *et al.*, 1978; J.L. Simpson *et al.*, 1981). XY females, who have small streak gonads like those of XO females, can be H-Y negative or positive (U. Wolf, 1979; Wachtel *et al.*, 1980; review in Meck, 1984), so that female differentiation may be due to a lack of expression of H-Y in the former case, and to H-Y receptor deficiencies in the latter (Wachtel *et al.*, 1980). There are clear parallels with the wood lemming case (Ohno, 1979), except that human XY females are sterile while wood lemmings are not.

A different kind of sex reversal (Sxr) occurs in mice, in which XX and XO individuals develop into sterile males whose germ cells have disappeared by adulthood (Lyon *et al.*, 1981). Mouse Sxr is inherited via a non-reciprocal transfer of the testis-determining locus on the tip of the Y chromosome to the distal part of the X chromosome during meiosis (Cattanach, 1985).

Familial XX sex reversal, which appears to be inherited as an autosomal dominant, occurs in humans (de la Chapelle, 1981), and Cattanach (1985) has argued that a genetic mechanism like the one operating in XXSxr mice may apply to humans as well. In isolated cases of XX sex reversal in man, patterns of inheritance (de la Chapelle *et al.*, 1984) and direct biochemical evidence (Guellaen *et al.*, 1984) do suggest that X–Y interchange occurs during meiosis, and that the amount of chromatin transferred may be variable (Cattanach, 1985).

The X chromosome and oogenesis

Two X chromosomes seem to be required for normal oogenesis in most mammals. One X can sustain development of the ovary, but is insufficient for its sustenance into adulthood and for oogenesis. XO females are usually infertile: in humans, for example, the streak gonads of such individuals are devoid of germ cells at adulthood (Carr *et al.*, 1968). In mice, XO females are fertile, but germ cells age unusually quickly (Carr *et al.*, 1968). Muller (1984) has related this difference to the different patterns of X chromosome inactivation in man and mouse. He suggests that X-linked genes promoting (or allowing) ovarian differentiation are among those that escape inactivation in man, in whom they must operate in double dosage, while mice require only one dosage. The need for double dosage in man evidently begins at meiosis, since only one X is active during oogonial proliferation (Vandeberg, 1983).

The X chromosome and spermatogenesis

The presence of two X chromosomes appears to block spermatogenesis. No fertile XX males have been reported in any mammal (Wachtel, 1986). XX germ cells in a testicu-

lar environment degenerate post-natally (McLaren, 1983). Germ cells with one X chromosome, however, may survive in a testis, as occurs in normal males. Even XO germ cells can survive in the testis of an XXSxr mouse (Lyon *et al.*, 1981). In XX/XY chimeric mice, XSxrO and XY germ cells even enter spermatogenesis, whereas XX germ cells are lost after birth (McLaren, 1983).

(c) Germ cell sex

In most normally developing individuals, the genotypes of germ cells and somatic cells are identical. There are reasons, however, for considering germ cell sex and gonadal sex separately. In females, germ cells, unlike XX somatic cells, do not undergo X inactivation during meiosis (McLaren, 1983). In males, germ cells, but not somatic cells, undergo X inactivation during meiosis. Furthermore, only male somatic cells express H-Y antigen, while germ cells do not. Finally, in some mammals, germ cell and somatic cell sex is regularly different. In the creeping vole (*Microtus oregoni*), for example, XY males produce only OY spermatogonia, while XO females produce only XX oogonia by non-disjunction and selective atresia. This example suggests that the Y chromosome serves a critical function only in the germ cells, where we have seen that it induces testicular organization.

Germ cells evidently do not contribute to the early organization of the gonad. If they are reduced in number or eliminated before arrival at the genital ridge, there is no effect on gonadal differentiation (review in Wachtel and Koo, 1981; Haseltine, 1983). Later, however, their presence may be required for normal gonadal differentiation: ovaries without oogonia do not develop normally (Wachtel and Koo, 1981).

There is evidence that the gonadal environment contributes to the sexual orientation of the germ cells. Zamboni and Upadhyay (1983) have argued that primordial germ cells are basically female: XX **and** XY mouse germ cells growing ectopically in the adrenal gland until 3 weeks post partum all differentiated into oocytes, closely following a typical mouse ovarian timetable. Thus, a testicular environment is necessary for XY germ cells to differentiate into spermatocytes.

A similar gonadal influence can operate on XO germ cells in mice. Normal XO mice are fertile females, though the oogonia undergo more rapid atresia than XX oogonia. XO sex-reversed mice, on the other hand, have testes, and their XO germ cells enter spermatogenesis (McLaren, 1983).

The degree to which the gonadal environment can influence germ cell differentiation is limited. In general, gonadal and germ cell sex must be compatible for normal development to occur (R.V. Short, 1982). No cases have been reported of XX germ cells successfully differentiating into testes in a testicular environment. In fact, in XXSxr mice there is evidence that they begin differentiation into oocytes which die before sexual maturity (McLaren, 1980).

6.6.2 SEXUAL DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM ('BRAIN SEX')

While the genes direct the initial determination of the gonads in early mammalian development, steroid hormones produced by these organs act later to influence the differentiation of secondary sexual characters such as behaviour. The fact that hormone manipulation around the time of birth can effect permanent changes in adult sexual behaviour, regardless of genotype and adult hormone treatments, suggests that such hormones have an organizing effect on the tissues mediating that behaviour, i.e. on the brain (Phoenix *et al.*, 1959). Such an effect differs from an activating effect, in that the presence or absence of certain hormones coincides with behavioural changes that are reversed when those hormones are removed or restored.

Table 6.9 Relation between the length of gestation and the timing of developmental critical periods for CNS-sexual differentiation (reproduced with permission from MacLusky and Naftolin, 1981)

Animal	*Gestation (days)*	*Critical period (after conception)*
Rat	20 to 22	18–27 days
Mouse	19 to 20	Post-natal
Hamster	16	Post-natal
Guinea pig	63 to 70	30–37 days
Ferret	42	Post-natal
Dog	58 to 63	Prenatal and post-natal
Sheep	145 to 155	~30–90 days
Rhesus monkey	146 to 180	~40–60 days

The organizing effects of early hormone exposure were first demonstrated on functional brain responses such as behaviour and the feedback system of pituitary hormones. More recent work has centred on the morphological correlates of hormone-mediated organization. Steroid-induced differences have been found in numerous areas of the brain, and the nature of the sex differences (e.g. neurone size, number and connectivity) is variable. The cellular mechanisms by which hormones cause morphological changes is currently an area of active research.

(a) Functional aspects of brain sex

Critical periods for hormone action

Studies of development of both brain function and structure have shown that there are limited critical periods for sexual differentiation of the brain. During these periods, brain structures and functions (that may appear later) are maximally susceptible to the permanent organizing effects of hormones, and these effects cannot be reversed by treatment at a later time. The timing of critical periods relative to birth varies between species (Table 6.9) but precedes eye opening in all cases that have been studied to date (Goy and McEwen, 1980).

Variation is due to the fact that birth occurs at various developmental stages in different species. Thus, in rats and guinea pigs, the critical period for hormone action spans a few days before and after birth, while in the relatively precocious rhesus monkey, the equivalent critical period spans several weeks during the first trimester of pregnancy (Goy and McEwen, 1980, Handa *et al.*, 1988; Sholl *et al.*, 1989). The temporal relationship between the critical periods of CNS development and gonad development is known only for a few rodents in which the critical CNS development seems to follow differentiation of the Leydig cells and onset of testosterone secretion.

The timing of critical periods also varies depending on the functional and structural parameters in question (Gorski, 1984). In guinea pig females treated with testosterone, for example, masculinization precedes defeminization, and the critical period for masculinizing behaviour begins sooner than that for masculinizing gonadotrophin regulation (Goy and McEwen, 1980). In the species studied so far, all of these reproductive functions, as well as brain structures, seem to have their critical periods of development before or around the time of birth, but sensitivity to

exogenous factors does not appear to be limited to this period (Gorski, 1986). However, organization of some non-reproductive sexually dimorphic brain functions (McEwen, 1983), such as taste preference in rats, occurs considerably later, i.e. at puberty (Shapiro and Goldman, 1981).

Sexual dimorphism of brain function

In normal female mammals, rising oestrogen levels trigger the cyclic release of gonadotrophin-releasing hormones from the hypothalamus and these, in turn, stimulate the release of two gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the pituitary gland. LH and FSH stimulate ovarian maturation and trigger ovulation. The positive feedback of oestrogen on gonadotrophin release is absent in normal males. However, males will not release LH or FSH in response to rising oestrogen or testosterone levels. Thus, the hormonal response of the pituitary and hypothalamus to oestrogen challenge is sexually dimorphic.

Sexual dimorphism is also apparent in the sexual behaviour of normal males and females (Goy and McEwen, 1980; Goy *et al.*, 1988). In rodents, the most frequently studied response by females to male mounting attempts is a concave arching of the back in the lordosis posture (Figure 6.12).

Lordosis is not part of the normal male's behavioural repertoire. They will, however, mount receptive females with thrusting while females typically do not perform this behaviour. In other mammals, the behaviour of receptive females takes different forms, while typical male behaviour usually includes mounting of receptive females. Other non-mating behaviour patterns are sometimes sexually dimorphic (e.g. urination in dogs, flehmen in sheep, taste preference in mice, various social situations in primates) and these have also been used as assays of functional organization of the brain (Quadagno *et al.*, 1977; Goy and McEwen, 1980; Pomerantz *et al.*, 1988). The degree to which sexually dimorphic behaviour is strictly sex specific varies from species to species (Goy and McEwen, 1980; J.J. Ford and D'Occhio, 1989).

Figure 6.12 Sexually dimorphic behaviour: lordosis in the female rat. Normal males do not perform this behaviour.

When sexual dimorphism involves different forms of a particular behaviour, rather than the absence or presence of the behaviour, it is useful to distinguish the masculinizing (or feminizing) effects of early hormone exposure from the defeminizing (or demasculinizing) effects (Beach *et al.*, 1972). As we shall see, the expression of typical female behaviour need not preclude male behaviour patterns, and vice versa (for review see Gorski, 1985a).

Effects of early exposure to hormones on development of sexually dimorphic brain functions

The response of both the developing and mature nervous system to hormones, particularly those secreted by the gonadal and adrenal glands, is related to the presence of receptor sites which mediate hormonal action at the level of gene expression (McEwen, 1988). When abnormal amounts of hormones are secreted, when they are secreted at the wrong time, or when exogenous hormones or

pseudohormones are ingested or absorbed, the nervous system may respond in abnormal ways. This may lead to permanent functional and structural changes in the brain which are associated with deviation or dysfunctions in reproductive function. These effects are generally more severe when experienced by the developing fetus than by the adult.

One of the important consequences of the developmental action of gonadal steroid is the programming of the male and female brain to respond differently to the same hormone (McEwan, 1988). Thus, the brain will respond differently to exogenous drugs and endogenous neurotransmitter depending on the individual's sex as well as the type of hormone present. The developing brain–pituitary–gonadal axis in both males and females is sensitive to abnormal concentrations of hormones or pseudohormones at critical developmental periods. For example, medroxyprogesterone acetate (MPA), a synthetic progestin, produces paradoxical genital abnormalities in cynomolgus monkeys, i.e. the external genitalia of female fetuses are masculinized and those of male fetuses are feminized (Prahalada *et al.*, 1985a). Adverse effects on psychosexual identity and other aspects of personality have also been associated with prenatal exposure to this drug in humans (Ehrhardt and Money, 1967; Reinisch, 1977). Thus, a basic understanding of the complex events that occur during normal sexual differentiation of the nervous system is a prerequisite to understanding the mechanisms which result in perturbed sexual development.

In rodents such as the rat and hamster, exposure to steroid hormones in late fetal life and in the first week of post-natal life plays a critical role in determining functional responses of the brain in adulthood. Exposure to androgen during this period leads to the development of masculinized behaviour and non-cyclic pituitary function. In the absence of androgen, female sexual behaviour patterns and cyclic gonadotrophin release occurs (review in Goy and McEwen, 1980; McEwen, 1983).

Because masculinization occurs prenatally, female rats from litters in which several littermates are male are much more likely to show mounting behaviour than females from litters with 0–1 male. The masculinization of female behaviour is greatest in those individuals located closest to males *in utero*, where androgen concentration is highest (Clemens and Coniglio, 1971; Clemens, 1974; Goy and McEwen, 1980; vom Saal, 1989). Similarly, females injected with androgens prenatally show masculinized behaviour (Ward, 1969).

Treatment with anti-androgens before birth blocks normal masculinization of males (Ward and Renz, 1972). There is some evidence that dihydrotestosterone (DHT) as well testosterone (T) is involved in masculinization (McEwen, 1983).

Defeminization of behaviour by androgens in rats follows masculinization and occurs during the first week after birth. This is also when androgens exert their strongest effect on gonadotrophin secretion (Arnold and Gorski, 1984). Female rats injected with testosterone propionate (TP) shortly after birth lose the lordosis response and do not show cyclic gonadotrophin release, while males castrated neonatally can show lordosis and do respond positively with gonadotrophin-releasing hormone after stimulation with oestrogen as adults (Dohler *et al.*, 1984a).

While these results appear to highlight the role of testosterone in perinatal organization of the rat brain, it has been found that oestradiol has similar effects, and is in fact more effective (per unit weight administered) than testosterone in preventing feminization of gonadotrophin release and behaviour (Gorski and Wagner, 1965; Sutherland and Gorski, 1972).

Non-aromatizable androgens such as DHT, on the other hand, are not effective in stimulating a male pattern of brain organization. A widely held hypothesis holds that testoster-

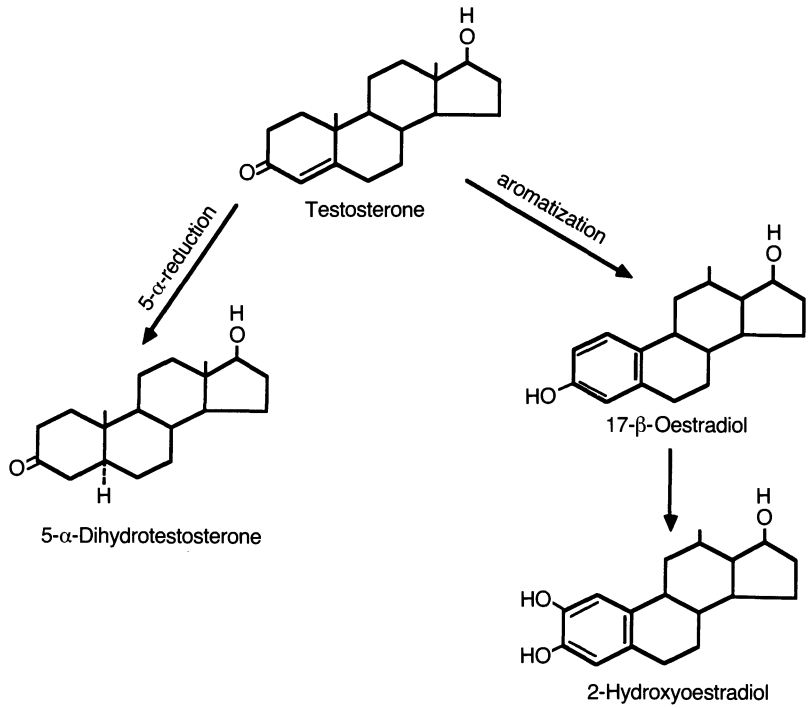

Figure 6.13 Transformations of testosterone that can occur in brain tissue.

one is converted in the brain via aromatization to the oestrogens oestrone and oestradiol, and that these are the active molecules (Figure 6.13) (MacLusky and Naftolin, 1981).

Since DHT cannot be transformed into oestrogen in the brain, it is ineffective in inducing male brain organization. This hypothesis is supported by the following observations: (i) androgen-to-oestrogen conversion does occur in the brain; (ii) anti-oestrogens block both testosterone- and oestrogen-induced masculinization; (iii) aromatase inhibitors block masculinization by testosterone; (iv) anti-oestrogens attenuate defeminization of females by testosterone; (v) in Tfm males, who lack androgen receptors, normal defeminization occurs in response to testosterone produced by the testes; and (vi) in cell cultures of mouse hypothalamus, neuritic outgrowth, which is typical of males, occurs only in cells whose oestrogen receptors are intact (Goy and McEwen, 1980; MacLusky and Naftolin, 1981; McEwen, 1983; Toran-Allerand, 1984a).

It is then necessary to explain why female rats are not masculinized by ovarian oestrogens which occur at high titres post-natally. It has been suggested that alpha-fetoprotein protects the developing female rat brain cells from excess oestrogens by binding them and thus keeping them in the circulation and out of the brain (Soloff *et al.*, 1972; Arnold and Gorski, 1984). Alpha-fetoprotein is present in large amounts only during gestation and immediately after birth, following the time course of neural differentiation (Toran-Allerand, 1984b). In rats and mice, it has a high affinity for oestrogen, but does not bind testosterone. As would be expected under the protection hypothesis, synthetic oestrogens which do not bind to alpha-fetoprotein

as well as oestradiol are more effective than oestradiol in inducing masculinization of the female rat brain (Goy and McEwen, 1980).

However, the alpha-fetoprotein protection hypothesis has been questioned. This high affinity of alpha-fetoprotein for oestrogen is not found in other species such as the guinea pig and human (MacLusky and Naftolin, 1981). Even in the mouse and rat, alpha-fetoprotein has been found within brain cells (Benno and Williams, 1978; Toran-Allerand, 1984b), though it is not made there in mice (Schachter and Toran-Allerand, 1982). Thus, alpha-fetoprotein may have other functions, including transport of oestrogen into the brain (Dohler *et al.*, 1984a; Toran-Allerand, 1984b). Other possible mechanisms for protection from oestrogen may exist, including placental barriers and progesterone-mediated anti-oestrogen action (McEwen, 1983).

Sex differences in fetal and neonatal testosterone titres are small or non-existent (Weisz and Ward, 1980); however, on days 18–21 post conception, titres are higher in males. Although these 4 days do not encompass the entire period of hormonal organizing action, the sex difference in testosterone levels during this time appears to be critical to sexual differentiation (Goy and McEwen, 1980). The higher testosterone levels in male fetuses at this time may exert a priming effect so that males are more sensitive to circulating testosterone later. The mechanism of priming could involve induction of the aromatizing enzyme system or of nuclear oestrogen receptors (Goy and McEwen, 1980; Toran-Allerand, 1984b; Goy *et al.*, 1988).

Androgenic induction of male functional brain organization has been most thoroughly studied in the rat, but this species is not typical (Table 6.10).

While masculinization and defeminization of brain function are distinct events occurring at different times and under the influence of different hormones in rats, defeminization has not been shown in ferrets, rhesus monkeys or humans. In these species, females treated with androgens during development have masculinized behaviour in addition to the normal female repertoire later in life, and cyclic gonadotrophin release with ovulation is retained (McEwen, 1983). Another primate, the marmoset, may show a reduction in female reproductive behaviour patterns when females are androgenized prenatally (Abbott and Hearn, 1979).

There is also variation among mammals in the pathway of androgen metabolism followed to produce the active molecule which induces masculinization or defeminization. In rats, both testosterone (or oestrogen) and DHT appear to be involved (McEwen, 1983). In guinea pigs and rhesus monkeys, however, prenatal treatment with DHT alone can organize some aspects of masculine sexual behaviour as effectively as does T (Goldfoot and van der Werff ten Bosch, 1975; Goy and McEwen, 1980). Why these interspecies differences exist is not known. Even the generalization that organizational and activational pathways are the same within a given species is not entirely correct, since some elements of male sexual behaviour in ham-

Table 6.10 Effects of perinatal administration of androgen on female development (Reproduced with permission from Goy and McEwen, 1980).

Species	*Dimorphic characteristic*[a]		
	Ovulation	*Female sexual behaviour*	*Male sexual behaviour*
Rat	↘↘	↘↘	↗
Mouse	↘↘	↘↘	↗↗
Hamster	↘	↘	↗↗
Ferret	0	0	↗↗
Dog	?	↘↘	↗↗
Guinea pig	↘↘	↘↘	↗↗
Sheep	↘↘	↘↘	↗↗
Rhesus	0	?	↗↗

[a] Arrows indicate direction and relative ease of obtaining effect:
↘ = defeminization; ↗ = masculanization; 0 = no effect

sters may be activated by non-aromatizable androgens which are not effective during development (McEwen, 1983).

(b) Structural aspects of brain sex

The study of anatomical measures of steroid hormone action began well after the studies of brain function (Arnold and Gorski, 1984). In a seminal paper, Raisman and Field (1973) documented a sex difference in the type of dendritic synapses in the preoptic area of the rat that could be reversed by manipulating neonatal androgen levels. Since then, there have been numerous reports of sex differences in central nervous system (CNS) anatomy, and perinatal exposure to steroids has been shown to affect axonal growth, dendritic connectivity (arborization), synapse formation, regional nuclear volumes and neuronal numbers in the hypothalamus, preoptic area, septum, amygdala and spinal cord (McEwen, 1983; Toran-Allerand, 1984a; L.S. Allen *et al.*, 1989), as well as a number of biochemical properties (McEwen, 1983) (Table 6.11).

For the most part, the functional significance of anatomical markers is not known, but they may provide useful models for understanding the mechanisms of hormone action on brain function.

Whereas many sexually dimorphic anatomical markers were discovered by direct inspection of brain tissue, localization of the circuitry for steroid-dependent organization of brain function has been investigated more indirectly as well. For example, by varying the site of oestrogen implants in newborn female rat brains, Nordeen and Yahr (1982) showed not only that masculinizing and feminizing implants must be placed in the preoptic area and ventromedial nucleus respectively, but also that behavioural masculinization results from implants in the right side of the hypothalamus, while defeminization of behaviour and gonadotrophin release follows implantation in the left side.

The sexually dimorphic nucleus of the preoptic area

A particularly well-studied sexually dimorphic brain structure, which is known to respond to early hormone exposure, is the sexually dimorphic nucleus (SDN) of the preoptic area (POA) of rats (Arnold and Gorski, 1984, Gorski, 1984). In males, this area of high- density neurones is about five times larger than in females (Figure 6.14).

Neuronal density is the same in both sexes, so the size difference must reflect a difference in cell number. Homologues for this area are also found in gerbils, ferrets and guinea pigs (Gorski, 1984). The exact function of the SDN-POA is not known, but the POA appears to play a complex role in many integrative processes. In rats, the POA appears to be critical for male copulatory behaviour. It is also involved in cyclic endocrine regulation in females (Gorski, 1984). In primates, on the other hand, the POA is not necessary for cyclic gonadotrophin release (Ayoub *et al.*, 1983).

A change in SDN-POA volume occurs simultaneously with functional masculinization of the brain in rats, though the volume does not correlate with the degree of expression of brain function (Gorski, 1984). SDN-POA size is not affected by gonadal steroids in adulthood. The size dimorphism develops within the first 10 days of life, when the adult SDN-POA size is achieved. It is hormones acting at this stage of development which influence SDN-POA size. In males castrated 1 day after birth, SDN-POA size is substantially reduced (Tarttelin and Gorski, 1988). If such males are treated with TP a day after castration, however, adult SDN-POA volume matches that of normal males. Female neonates injected once with testosterone propionate or oestradiol benzoate show increased SDN-POA volume as adults. If TP administration is prolonged, adult SDN-POA volume of females may be fully sex reversed (Gorski, 1984).

Table 6.11 Sex differences in the brains of infrahuman mammals (Reproduced with permission from McEwen, 1983)

Description	*Region*[a]	*Species*	*Org?*[b]
Morphological			
1. Size of nerve cell nuclei and nucleoli	Hyp, POA, Hippo, cortex, Hab	Rat	Yes
2. Size of nerve cell nuclei	Hyp, POA	Rat	Yes
3. Size of nerve cell nuclei	Amygd	Squirrel monkey	?
4. Size of pyramidal neurones	Cortex	Rat	?
5. Ratio of synaptic 'types'	POA	Rat	Yes
6. Dendritic distribution	POA	Hamster	Yes
7. Size of neuroanatomical 'nucleus'	POA	Rat	Yes
8. Size of neuroanatomical 'nucleus'	Spinal cord	Rat	Yes
9. Dendritic branching	POA	Macaque monkey	?
10. Synaptic 'type'	Hyp	Rat	Yes
11. Synaptic 'type'	Amygd	Rat	Yes
12. Bungarotoxin-binding region	Amygd	Mouse	Yes
13. Vasopressin neurones	Septum, Hab	Rat	?
14. Response to septal damage	Hippo	Rat	Yes
15. Neurovascular contact surface	Hyp	Rat	?
16. Lateralization of 2-deoxyglucose uptake	Many areas of brain	Rat	?
17. Lateralization of cortical thickness	Cortex	Rat	Yes
Biochemical			
18. Hypothalamic peptidases	Hyp	Rat	Yes
19. Cholinergic enzymes	POA	Rat	Yes
20. Cholinergic enzymes	POA, Amygd	Rat	?
21. Monoamine oxidase	Hyp, Amygd	Rat	?
22. Noradrenaline, dopamine levels	Many areas	Rat	?
23. Dopamine levels	Hyp, POA	Rat	Yes
24. Neurotransmitter enzymes	Many areas	Rat	?
25. Trace element levels	Hyp, pit	Rat	?
26. Glutathione-*S*-transferase activity	Whole brain	Rat	?
27. Muscarinic receptors	POA, pit	Rat	?
28. Glucocorticoid receptors	Hippo	Rat	?
29. Serotonin level in early development	Whole brain	Rat	Yes*
30. Monoamine oxidase activity in early development	Hyp	Rat	Yes*
31. Serotonin synthesis on post-natal day 2	Whole brain	Rat	?
32. Serotonin synthesis in early development	Whole brain	Rat	Yes*
33. Serotonin, noradrenaline levels in early development	Various areas	Rat	Yes*
Functional			
34. Amygdala projections to preoptic area (electrophysiology)	Amygd, POA	Rat	Yes
35. Release of dopamine, noradrenaline *in vitro*	Caudate, Hyp	Rat	?
36. Amphetamine-elicited rotational behaviour		Rat	?

It appears that SDN-POA size is actually determined by oestrogen, rather than by testosterone. Thus SDN-POA size is the same in Tfm rats which lack androgen receptors as in normal males. Also, diethylstilboestrol, which does not bind to alpha-fetoprotein, can cause complete sex reversal of SDN-POA size in females if administered throughout the perinatal period, just as does TP (Dohler *et al.*, 1984b).

The spinal nucleus of the bulbocavernosus muscle

A second well-studied sexually dimorphic part of the central nervous system that responds to perinatal gonadal hormones is the spinal nucleus of the bulbocavernosus muscle (SNB) in rats (Arnold and Gorski, 1984, Breedlove, 1984).

In males, this nucleus is an easily visible compact structure, while in females it contains only a third as many motoneurones, which are smaller (Figures 6.15 and 6.16).

The SNB system has an advantage over the SDN-POA system in that the functional correlates of the morphological dimorphism are known. The SNB, located in the spinal cord, consists of motoneurones innervating the levator ani (LA) and bulbocavernosus (BC) muscles in males. In rodents, these muscles occur only in males, where they attach to the base of the penis and mediate penile reflexes important in copulatory plug formation, which ensures effective copulation (Breedlove, 1984).

Unlike the SDN-POA, sexual dimorphism of the SNB in rats is mediated by androgens and not by oestrogens. This clearly indicates that sexual differentiation of brain morphology occurs by different mechanisms depending on the particular structure. SNB motoneurones are unable to accumulate oestrogens, but do accumulate DHT and testosterone. However, only the accumulation of testosterone is sexually dimorphic. In addition, there are androgen receptors in the levator ani muscle (Jung and Baulieu, 1972), so that the muscles themselves must be considered as a potential site of hormone action, along with or instead of the motoneurones (Breedlove, 1984).

Tfm males lacking androgen receptors have female-type SNBs and lack the levator ani and bulbocavernosus muscles. Similarly, when normal males are treated with antiandrogens prenatally and castrated as neonates, their adult SNB system is completely sex reversed. If females are treated with testosterone propionate or DHT propionate (DHTP) perinatally, the SNB system is masculinized. However, masculinization does not occur if oestrogen is used, even in higher doses (Breedlove, 1984).

(c) Mechanisms of sexual differentiation of the central nervous system

The discovery of hormone-sensitive morphological parallels of sexually dimorphic brain functions has focused attention on cellular mechanisms of sexual differentiation which affect the structure of neural circuits and, thus, ultimately lead to dimorphic brain function. Experimental manipulations have shown that hormonal action of CNS sexual differentiation depends both on the time course of exposure to hormones (see next section on critical periods below) and on the dose of hormone administered (Toran-Allerand, 1984a; Handa *et al.*, 1985; Sholl *et al.*, 1989).

Morphological dimorphism could result from hormonal effects on the rate or period of neurogenesis, on the pattern of neuronal differentiation, on cell migration patterns, or on cell survival. These effects may be direct, in that hormones act on the dimorphic neurones themselves, and/or indirect, in that the targets and afferents of those neurones are affected (Arnold and Gorski, 1984). Gonadal hormones may influence several developmental processes directly, or may trigger one such process which affects many others in a cascading manner (Figure 6.17)

Table 6.11 *continued*

Description	*Region*[a]	*Species*	*Org?*[b]
37. Electrical stimulation of rotational behaviour		Rat	?
38. Lateralization of dopamine levels	Caudate	Rat	?
39. Turning preferences and postural asymmetry		Rat	?
40. Amphetamine-stimulated activity		Rat	?
41. Haloperidol-induced catalepsy		Rat	?
42. Release of dopamine into hypophyseal portal blood	Hyp	Rat	?
43. Activity of tuberoin-fundibular dopamine nerones	Hyp	Rat	Yes
44. Sensitivity of tuberoin-fundibular dopamine neurones to prolactin	Hyp	Rat	?
45. Electrical responses of neurones to oestradiol and testosterone	Hyp, Amygd, Hippo	Rat	?
46. Behavioural and thermal response to pargyline and tryptophan		Rat	?

[a] Abbreviations: Amygd, amygdala; caudate, caudate nucleus; Hab, habenula; hippo, hippocampus; Hyp, hypothalamus; Pit, pituitary; POA, preoptic area.
[b] Org? – Is there evidence for an organization effect? ?, unknown; Yes, some positive evidence available; Yes*, sex differences are transient during development.

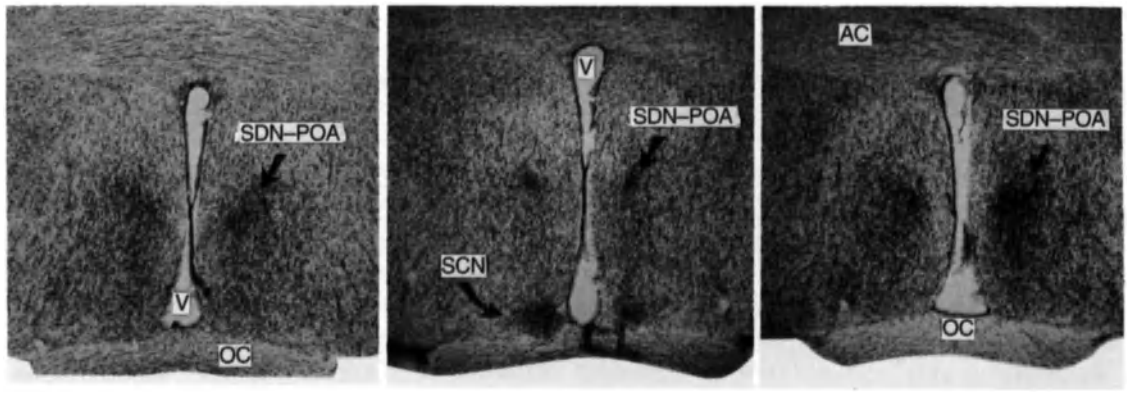

Figure 6.14 Photomicrographs (at same magnification) of coronal sections through the rat brain at the level of the centre of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the male (A), female (B) and the female exposed to testosterone propionate (TP) for a prolonged period during perinatal development (C). AC, anterior commissure; OC, optic chiasm; SCN, suprachiasmatic nucleus; V, third ventricle. (Reproduced with permission from Gorski, 1983.)

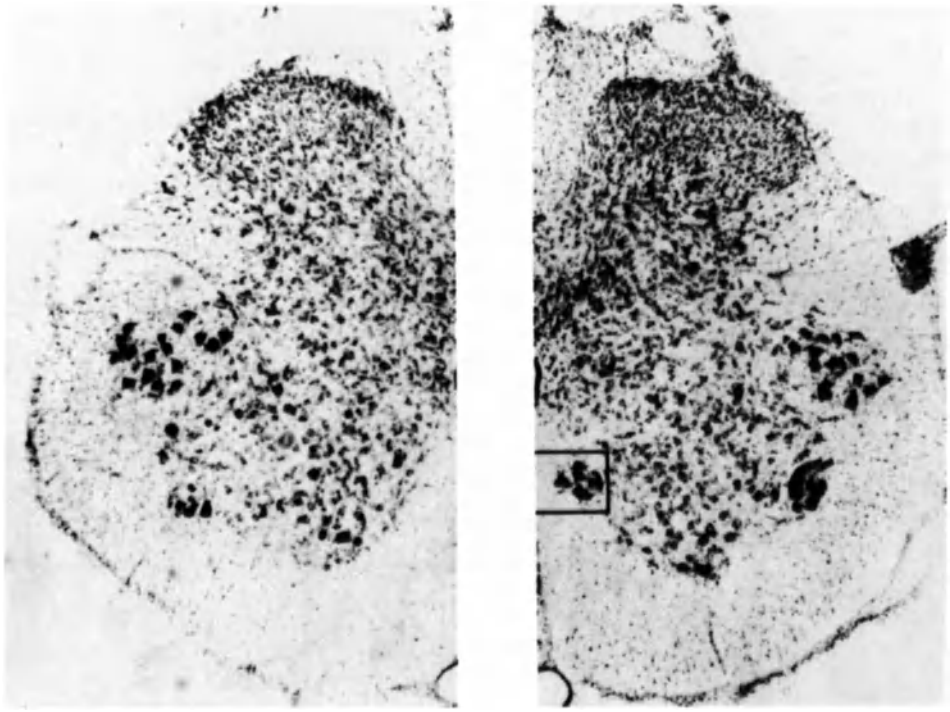

Figure 6.15 Photomicrographs of the fifth lumbar segment of the spinal cord of a female (left) and male rat. The spinal nucleus of the bulbocavernosus (SNB) is an easily recognized, discrete nucleus in the male only. The arrows indicate the anatomical limits of the SNB in males (right). This region, 200–400 mm ventral to the central canal and within 250 mm of the midline, was examined in male and female fifth and sixth lumbar spinal segments, within which all densely staining cells were designated SNB cells for comparison between treatment groups and the two sexes. (Reproduced with permission from Breedlove, 1984).

(Arnold and Gorski, 1984; Toran-Allerand, 1984b).

Data on the development of the SDN-POA and SNB suggest that the first hypothesis, differential neurogenesis, is unlikely since neurogenesis stops before the hormones are present and can exert their effects (Breedlove, 1984; Gorski, 1984). The possibility that hormones act to redifferentiate neurones has also been discounted for SNB development since labelling experiments show that motoneuronal differentiation occurs before androgen-induced SNB formation (Breedlove, 1984). Hormonal influence on migration patterns has been proposed to account for sexually dimorphic SDN-POA development, and current evidence suggests that this is a possible explanation: for example, steroid hormones may influence neuronal death before neurones finish their migration to the SDN-POA (Gorski, 1984). The hypothesis that hormones mediate cell death is perhaps the most widely favoured, although evidence is circumstantial. It is generally accepted that

more neurones are produced than survive development, that survival depends on the establishment of appropriate connections, and that the growth of neuronal processes can be stimulated by oestrogen (Gorski, 1984). Thus, hormonal mediation of cell death would occur indirectly via the effects of hormones on neuronal development. Supporting this hypothesis is the observation that the time course of (non-SNB) motoneurone cell death coincides with sexual differentiation of the SNB. Studies are currently under way in which the incidence of dying (pyknotic) SNB cells in relation to SNB development is examined.

Steroid hormones may act on biochemical features of neurones as well as morphological ones (McEwen, 1983). For example, the expression and concentration of neurotransmitters may differ between the sexes. Such biochemical differences may lead to differential sensitivity of male and female neural tissue and enzyme systems to hormones later in life.

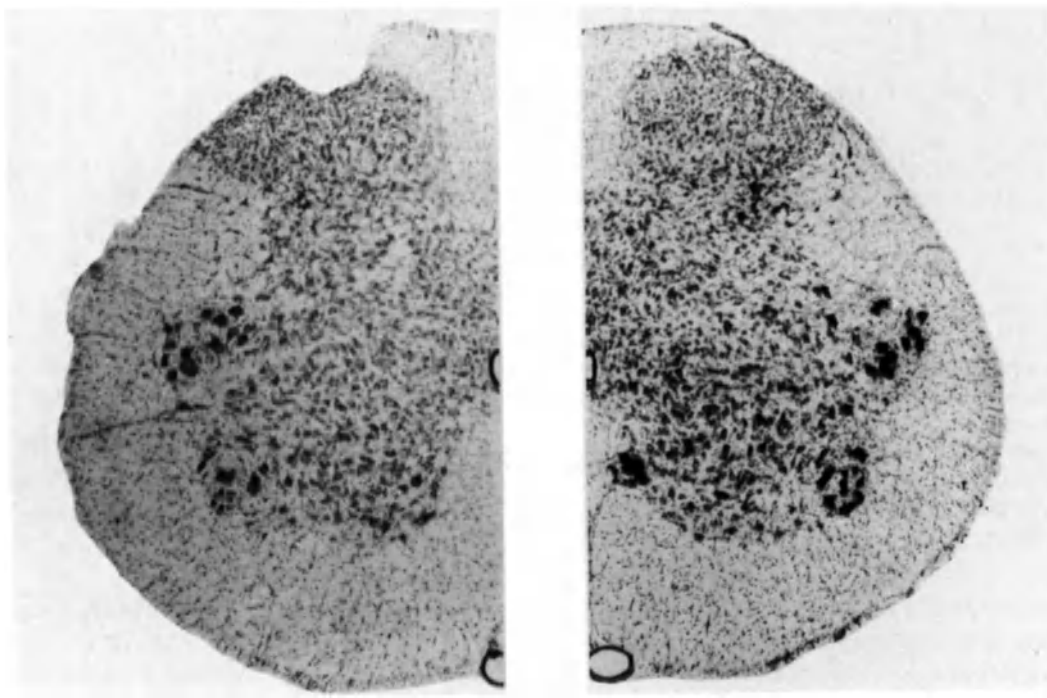

Figure 6.16 Photomicrographs of transverse sections of the fifth lumbar spinal segment from a male (left) and female rat. The arrows point out the anatomical region occupied by the SNB in normal male rats. The spinal cord on the left lacks a coherent nucleus because this male was treated prenatally with an anti-androgen and was then castrated at birth. The spinal morphology is within the range of normal female rats in this male which, like females, does not possess the SNB target muscles BC/LA. The spinal cord on the right is from a female which was injected with 1 mg of DHTP on days 1, 3 and 5 of life. In adulthood, this female possesses an SNB which innervates the muscles BC/LA. (Reproduced with permission from Breedlove, 1984.)

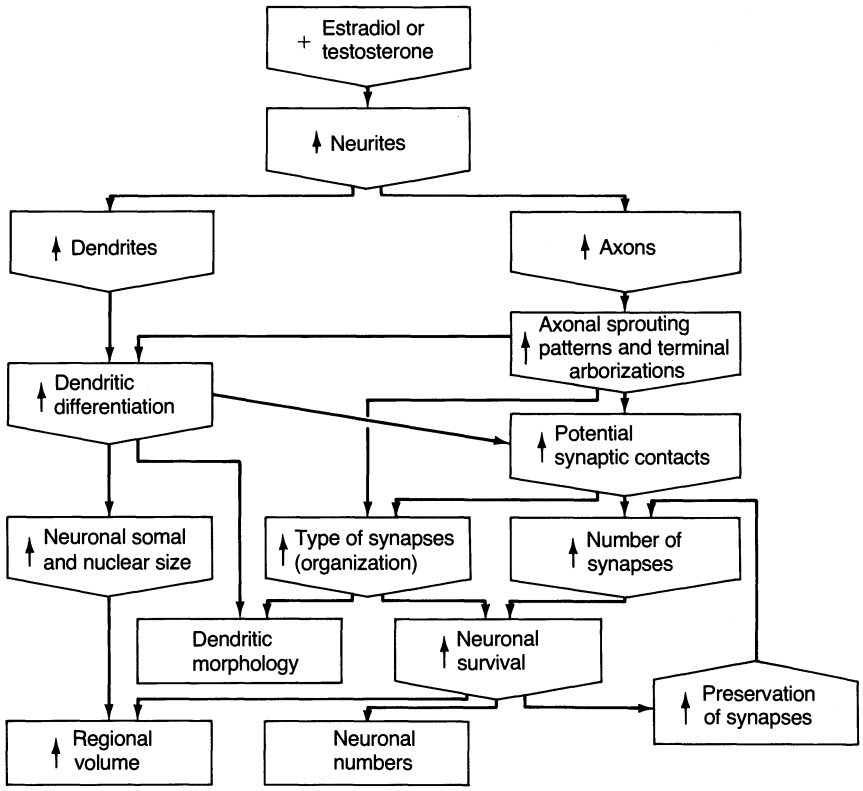

Figure 6.17 The cascade hypothesis. Morphogenetic consequences of early stimulation of neuritic growth by steroid could elicit a cascade of cellular events affecting subsequent aspects of target neural differentiation to result in the sexual dimorphism of brain structures. Different scenarios are also possible (Arnold and Gorski, 1984). (Reproduced with permission from Toran-Allerand, 1984b.)

Regardless of exactly which changes in neural anatomy and biochemistry are affected by steroid hormones in development, it is clear that the hormone receptors are a critical component of the system. The identity and concentration of these receptors in early development varies with time, location in the brain and sex (i.e. exposure to hormones) (Figure 6.18) (McEwen, 1983; Sholl *et al.*, 1989).

Furthermore, dimorphic receptor profiles in adults, which may lead to dimorphic brain function, have been shown to depend on perinatal exposure to particular hormones at particular times (Rainbow *et al.*, 1982; Weiland and Barraclough, 1984; Gorski, 1985b).

(d) Female differentiation of the CNS: a default process?

Although female organization of the CNS is often assumed to occur in the absence of stimulation of male differentiation, there is evidence that the organization of the female brain is also an active process requiring exposure to steroid hormones. Roles for testosterone (Gorski, 1984) and progesterone (Shapiro and Goldman, 1981) have been suggested, but most emphasis has been

placed on the role of oestrogen (Dohler *et al.*, 1984a; Toran-Allerand, 1984b). Dohler *et al.* (1984a) have criticized the assumptions on which the 'female by default' hypothesis was based. According to these authors, and contrary to what is widely assumed, oestrogen is available to the developing nervous system of female rats, either from the reservoir bound by alpha-fetoprotein (which can be found within brain cells) or from the mother. In addition, large numbers of oestrogen receptors are present in certain CNS regions where aromatizing enzymes are absent (Toran-Allerand, 1984b).

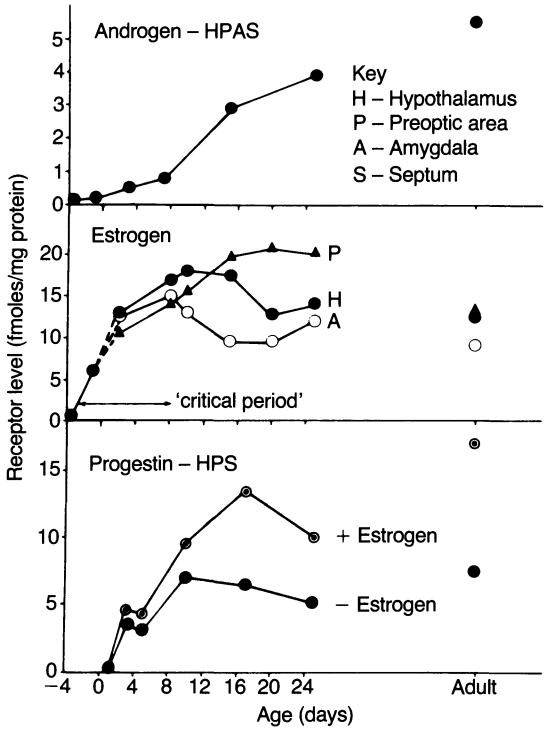

Figure 6.18 Developmental time course of gonadal steroid receptors in female rat brains. Receptor levels are expressed as fmol per mg of cytosol protein, are plotted against age in days before or after birth. Brain structures are denoted by letters (see key); note that they were studied individually (oestradiol) or as tissue pools (e.g. hypothalamus-preoptic area-septum-amygdala (HPAS) and hypothalamus-preoptic area-septum (HPS)). Androgen receptors were measured with [^{3}H]testosterone and were isolated by DNA–cellulose chromatography. Oestrogen receptors were measured with [^{3}H]R-2858 (moxestrol) by the Sephadex LH-20 gel filtration method. Progestin receptors were measured with [^{3}H]R-5020 by the LH-20 method. Arrows denote critical period for defeminization. (Reproduced with permission from McEwen, 1982.)

Direct evidence supporting the role of oestrogen *per se* comes from cases where oestrogen levels during development were experimentally reduced (Dohler *et al.*, 1984a; Toran-Allerand, 1984b):

1. Females treated post-natally with tamoxifen, an oestrogen antagonist, develop permanent anovulatory sterility, without masculine behaviour.
2. The presence of ovaries post-natally is known to influence feminization of receptive behaviour later in life.
3. Ovariectomy of neonatal females, but not adult females, changes the asymmetry in the cerebral cortex to a typical male pattern.
4. Neonatally gonadectomized males and females develop female-type non-reproductive behaviour when treated with small (sub-masculinizing) amounts of oestrogen.
5. Cultured female hypothalamic cells show abnormally reduced neuritic development when the culture medium lacks oestrogen.

If oestrogen does play an active role in female differentiation of the brain, the question remains how this one hormone can induce both feminization and masculinization. Dohler *et al.* (1984a) have proposed a quantitative effect which states that moderate levels of oestrogen, provided by maternal and placental tissues and bound to alpha-fetoprotein, cause female development of the brain. High levels of oestrogen, derived from aromatization of androgen (and perhaps acting synergistically with androgen), induce masculinization. An even higher threshold must be met for defe-

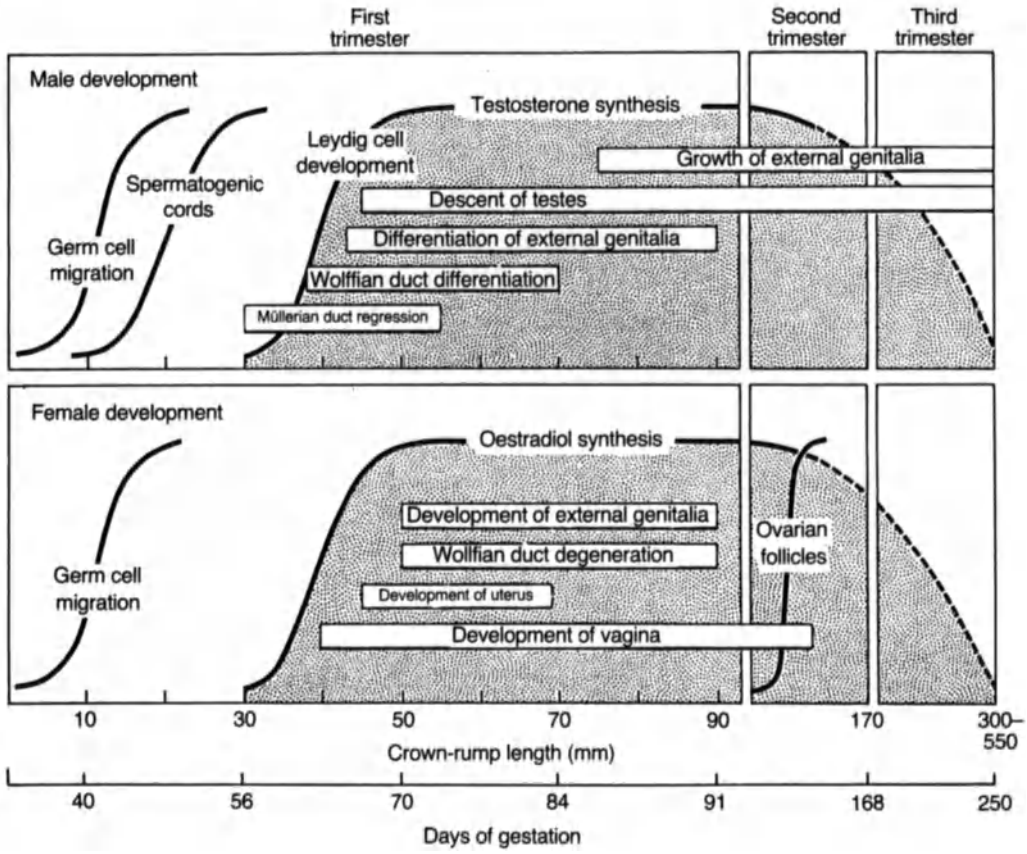

Figure 6.19 Relation between differentiation of the gonads and the anatomical differentiation of human male and female embryos. (Reproduced with permission from J.D. Wilson *et al.*, 1981.)

minization. This quantitative effect would depend not only on the titre of oestrogen at any one time, but also on the duration of its presence during critical periods of development.

6.6.3 MORPHOLOGY OF SEXUAL DIFFERENTIATION

In most instances, differentiation of the genital tract proceeds craniocaudally, thus affecting the gonads and the ducts first and the external genitalia last. One notable exception to this is in the bovine fetus, where the onset of penile development is the first sign of male differentiation (Vigier *et al.*, 1976). Figure 6.19 summarizes some of the morphologic features of the developing reproductive system in the human.

(a) Gonads

Even though the genetic sex of an individual is determined at fertilization, the reproductive systems of both sexes go through an early indifferent stage of development during which time the gonads remain morphologically indistinguishable until testicular differentiation occurs (Gondos, 1985). It is generally accepted that the indifferent gonad is a consortium of somatic and germinal cells composed of primordial germ cells, which (according to most human embryology textbooks) migrated from the yolk sac to the future gonadal ridge (Witschi, 1963). More

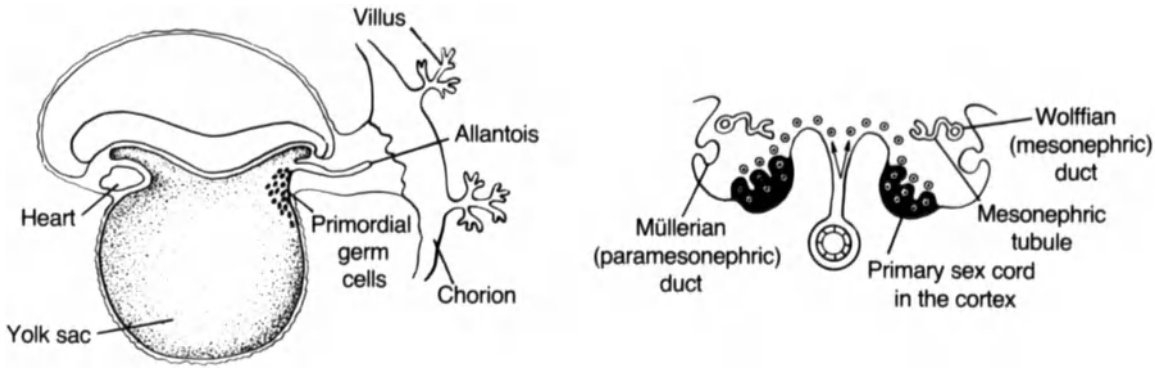

Figure 6.20 Schematic drawing of a 3-week human embryo (left) showing the primordial germ cells in the wall of the yolk sac, close to the attachment of the allantois. Schematic drawing of a transverse section through a 6-week human embryo (right) showing the primary sex cords and the developing mesonephric and paramesonephric ducts.

recent evidence from the mouse indicates that the primordial germ cells are derived from the epiblast, at the posterior end of the primitive streak (McLaren, 1992). Here they intermix with epithelial cells and underlying mesenchymal cells of mesodermal origin (Figure 6.20).

The origin and lineage of somatic cells contributing to the development of the gonad remained a point of interest and controversy for many years (Jost, 1985; Wartenberg, 1985). There is a large body of data that supports the view that epithelial cells are derived from the coelomic epithelium,which forms a layer of cells over the gonadal ridge (K.L. Moore, 1982; Sadler, 1985; Yoshinaga *et al.*, 1988). However, there are several studies in the mouse, rabbit and sheep which indicate that the blastema cells of the gonad have a nephric origin (Fraedrich, 1979; Kinsky, 1979; Upadhyay *et al.*, 1979; Wartenberg, 1979, 1981; Zamboni *et al.*, 1979).

The primordial germ cells are large, spherical to ovoid cells that can be identified histochemically by their high alkaline phosphatase and glycogen content. The presence of the primordial germ cells in the newly formed gonadal ridge stimulates the coelomic epithelium to multiply and invade the developing gonad. Cords of cells, called the sex cords, containing epithelial cells and primordial germ cells, form and give rise to the testis and ovary.

In the male gonadal ridge, under the influence of the Y chromosome, the contact between the primordial germ cells and somatic cells occurs earlier than in the female gonadal ridge and leads to formation of seminiferous tubules rather than follicles (Jirasek, 1976, 1977; Ohno, 1979). This occurs between 42 and 52 days of gestation or developmental stages 18–20 (O'Rahilly, 1983) and is thought to be mediated by the H-Y antigen, a gene product of the Y chromosome. It coincides with the acquisition of anti-Mullerian activity of the male gonad (Tran *et al.*, 1977). The process of gonadal differentiation and reproductive tract development has been studied most extensively in the human. More limited studies in several other species, i.e. dog, mouse, rat, rabbit, sheep, suggest that the basic processes are similar in mammals.

Male

In the human, during the seventh week of development, the presence of the male sex chromosome causes the genital ridge to se-

crete testosterone, which induces testicular development as well as development of other internal and external reproductive organs (Saenger, 1984; Gondos, 1985). At the same time, the development of the female genital organs is suppressed. Because of the proliferation of the mesenchyme the sex cords of the genital ridge become separated from the adjacent coelomic epithelium. The tunica albuginea, a fibrous layer located between the coelomic epithelium and the sex cords, forms from a condensation of the newly formed mesenchyme. During the fourth month in the human the U-shaped sex cords form the seminiferous tubules. The free ends of these tubules, the straight tubules, join together to become the rete testis. After canalization, the tubules join with the remnants of the mesonephric tubules, the future efferent ductules of the testis. The remaining part of the internal duct system, the epididymis, the vas deferens, the seminal vesicles and the ejaculatory ducts are formed from the mesonephric duct.

The primordial sex cells in the seminiferous tubules form the spermatogonia and the sex cord cells give rise to the Sertoli cells (Tran *et al.*, 1977). Both H-Y antigen and anti-Mullerian hormones are secreted by the Sertoli cells. Initially, groups of primordial germ cells and differentiating Sertoli cells become surrounded by a basal lamina within the fetal testis. At this time the germ cells are randomly distributed among the numerous Sertoli cells (Gondos and Hobel, 1971). The interstitial cells or Leydig cells, which are already secreting testosterone, are mesenchymal in origin. Leydig cells first appear at about 60 days of gestation, and by 14–18 weeks make up more than half the volume of the testis (Pelliniemi and Niemi, 1969). Seminiferous tubules remain as solid cords until puberty; at that time, in response to stimulation by the pituitary gonadotropic hormone, there is a proliferation of spermatogonia, an increase in size of the Sertoli cells and a lumen is formed in the tubules.

Jost (1972) and Jost *et al.* (1973) found that the first event to occur in the differentiation of the rat testes was the emergence of the primordial Sertoli cells, which then aggregated into seminiferous cords. This process of aggregation occurred in a period of a few hours, between 13 days 7 hours and 13 days 23 hours. Both electron microscopic and *in vitro* studies confirmed this initial observation (Magre and Jost, 1980, 1984; Magre *et al.*, 1981; Agelopoulou *et al.*, 1984). A similar process of seminiferous cord formation occurs in rabbits (Jost *et al.*, 1985).

Female

In the female the absence of testosterone from the fetal testes induces the development of the ovary and other female genital organs. The female gonad differentiates later than that of the male (Figure 6.19); the first morphological signs of ovarian differentiation occur in the human in the eighth gestational week. The sex cords are broken up into cell clusters by the proliferating mesenchyme. However, a thick tunica albuginea does not form as in the male. The primordial germ cells differentiate into oogonia, which undergo mitotic division to form primary oocytes beginning in the third month of pregnancy. The primary oocytes are surrounded by a single layer of cells, the granulosa cells, which are derived from the epithelial cells of the sex cords. The primary oocytes, together with the surrounding granulosa cells, are known as primordial follicles. It is believed by some authorities that many of the primordial follicles are killed and that a secondary proliferation of sex cords from the coelomic epithelium occurs. These sex cord cells again become associated with pre-existing primordial germ cells, and additional primordial follicles are formed. The ovarian stroma is derived from the mesenchyme surrounding the follicles.

The morphologic development of the human fetal ovary is much slower than that

of the testis. By 20 weeks of fetal age, more than 3 months after the start of testicular development, the ovarian germ cell population reaches a peak of approximately seven million cells. This population then decreases to two million cells at term as a result of atresia and of cessation of mitotic activity (T.G. Baker, 1963).

The mechanisms responsible for gonadal sex differentiation are still unknown (J.D. Wilson *et al.*, 1981; Saenger, 1984; Gondos, 1985). Since testicular differentiation precedes ovarian differentiation in all mammalian species studied so far, a specific inductive factor in the male may be responsible. However, the identity of such a factor, or factors, has not been determined, although there is evidence that Sertoli cells may trigger testicular development without further development of the Y chromosome (Burgoyne, 1988)

(b) Duct system

In the undifferentiated state two sets of paired ducts, the Wolffian and Mullerian ducts, are present in all embryos of either sex, and will give rise to major portions of the male and female reproductive tracts (Figure 6.21).

Male

Differentiation of the duct system in the male is characterized by regression of the Mullerian ducts, retention of the Wolffian ducts and their subsequent differentiation. The primordia for male sex accessory organs, particularly the Wolffian ducts, originate from the embryonic kidney, the mesonephros. The contribution from the mesonephros is incorporated into the genital system after the definitive kidney, the metanephros, develops.

In the human, the Wolffian ducts develop dorsolateral to the pronephric blastema, the forerunner of the mesonephros, and lateral to somites 8–13 (Torrey, 1954). They grow caudally, reaching the hindgut (cloaca) in human embryos at 28–30 gestational days (GD). The mesonephric nephrons and caudal tubules degenerate but the cranial mesonephric tubules are retained as the vasa efferentia, which connect the rete testis to the epididymis. The portion of the Wolffian duct that becomes the epididymis undergoes extensive lengthening and convolution. Later the distal segment of the Wolffian duct develops a thick muscular layer and becomes the ductus (vas) deferens (Figure 6.21).

Only limited information on the cellular differentiation of the male reproductive system is available. At the ultrastructural level, in the differentiating rat epididymis, cisternae of the granular endoplasmic reticulum form numerous specializations resembling intracytoplasmic confronting cisternae (Flickinger, 1969). The apical cell surfaces form numerous cylindrical microvilli, separated by areas covered by a thickened plasma membrane and dense material applied to its cytoplasmic side. Agranular endoplasmic reticulum increases in the apical region of the cell. In the sheep embryo, cilia appear in the epididymis near term and maturation is marked by an increase in the size of the Golgi complex and in the glycogen content of the cells. Lysosomes are also very common (Tiedemann, 1971). Mucus secretion from the epididymis is evident in the human fetus from 25 weeks of gestation onwards (Zondek and Zondek, 1965).

The seminal vesicle originates as a dilation of the terminal portion of the vas deferens in 70-day human fetuses (Jirasek, 1971) and in 18- to 19-day-old rat fetuses (Flickinger, 1970). The part of each Wolffian duct between the seminal vesicle and the urethra becomes the ejaculatory duct. In the rodent, development of the seminal vesicle is completed post-natally and is characterized by the appearance of primary and secondary epithelial cell branching and the abundant deposition of glycosaminoglycan cell matrix

in the cell membrane between the branches (Cunha and Lung, 1979).

The fine structure of the undifferentiated Wolffian duct has been described in the rat (Flickinger, 1969), the mouse (Dyche, 1979) and the sheep (Tiedemann, 1971) and more recently in the human (Lawrence *et al.*, 1992). The columnar cells, which make up the duct walls, contain moderate amounts of granular endoplasmic reticulum, sparse agranular endoplasmic reticulum and many dense bodies and lysosomes.

The onset of Mullerian duct degeneration in the male embryo begins at the end of the bisexual stage of development. In the human embryo, at 55–56 gestational days (GD), the first signs of regression appear in the area where the ducts are closest to the caudal pole

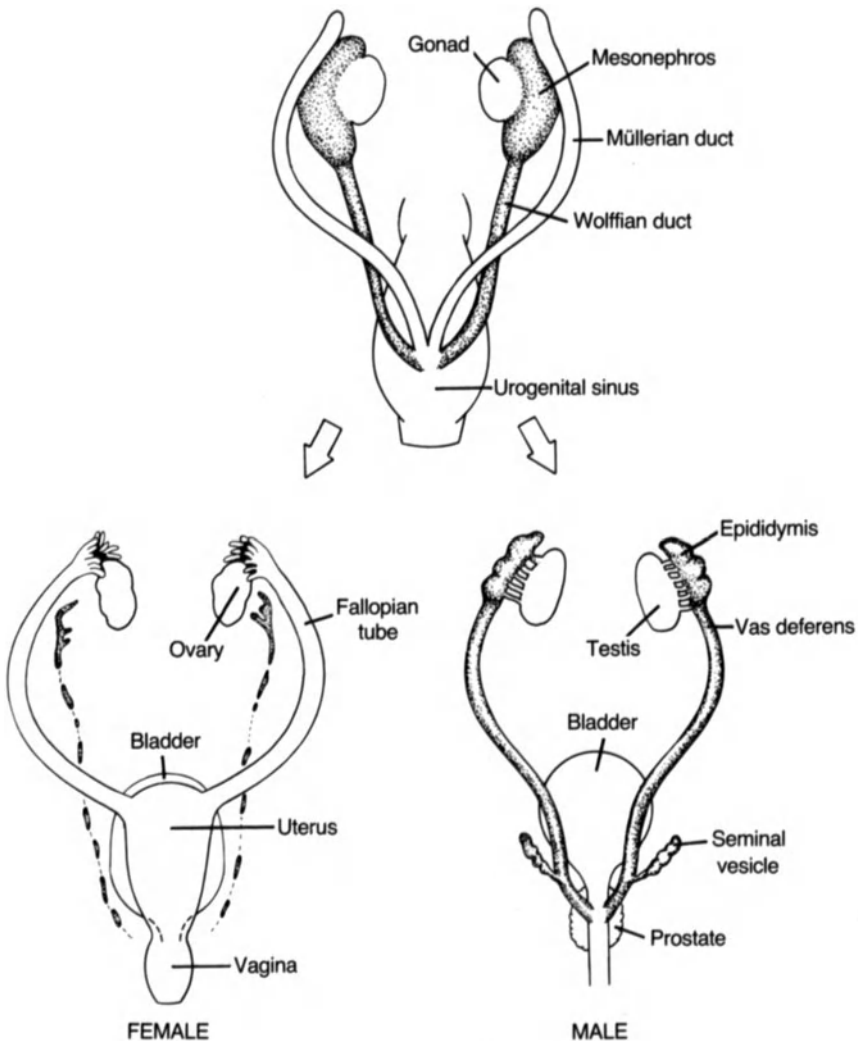

Figure 6.21 Development of the male and female internal genitalia from the Mullerian and Wolffian ducts of the indifferent stage. In the male, the prostate is an outgrowth of the urogenital sinus and is not derived from the Wolffian duct. In the female, the lower part of the vagina is formed from the urogenital sinus.

of the testis and cross the caudal ligament. This ligament is invaded by an acid mucopolysaccharide substance secreted by the fetal testis (Jirasek, 1977). Regression progresses in a craniocaudal sequence, sparing only the cranial portion, which persists to form the appendix testis, and the caudal tips, which remain as vestigial structures, the prostatic utricle (Figure 6.22).

In the human embryo the Mullerian duct is completely regressed in embryos 60–62 GD and in the rat by GD 20. While it is recognized that extensive cytolysis is a normal feature of both Mullerian and Wolffian duct development, the extrusion of whole degenerated cells from the duct early in regression appears to be unique to the involution of the Mullerian duct. Involution may also induce a process called 'puffing' in the periductal mesenchymal cells, which become joined by junctions resembling desmosomes. Their morphology is different from those mesenchymal cells surrounding the duct earlier in gestation.

Female

Female sex differentiation lags behind that of the male (Figure 6.19), although sex determination occurs at the same time in both sexes. Major events affecting the function of the female reproductive organs occur during fetal development. For example, 8-week-old human female Mullerian ducts, which are nearly fully formed, no longer respond to anti-Mullerian hormone. It is at this exact time that the Wolffian ducts begin to degenerate in female embryos (Josso *et al.*, 1977). Similar responses are observed in the 15-day rat fetus (Picon, 1969). The Wolffian ducts contribute mesenchymal tissue to the uterine wall (Witschi, 1970) and the lower portions contribute to vaginal formation (Forsberg, 1965).

Female genital development in mammals is characterized by retention of the Mullerian ducts and their subsequent differentiation into Fallopian tubes, uterus and upper vagina (Figure 6.21). In the 37–40 GD human embryo, Mullerian or paramesonephric ducts first appear as clefts lined by coelomic epithelium lateral and parallel to the Wolffian ducts. Caudal to this cleft, a solid bud of epithelial cells burrows progressively into the mesenchyme in a caudal direction. By the eighth week in the human the solid tip of the Mullerian ducts lies in the pelvis and has crossed ventral to the Wolffian ducts to assume a medial position. As the epithelial bud increases in length, a lumen first appears in its cranial-most end, where it is continuous with the coelomic cavity, and gradually extends caudally until the paired ducts reach the urogenital sinus. For a short time two lumina persist but the medial septum, under normal conditions, disappears shortly after fusion. The unfused cranial portions of the Mullerian ducts become the paired Fallopian tubes and the fused portions become the uterus and upper vagina. The caudal tips of the Mullerian ducts, the Mullerian tubercle, cause an elevation of the dorsal wall of the urogenital sinus and give rise to sinusal tissue. Mullerian ducts do not open into the urogenital sinus until much later in development, when canalization of the vagina occurs. In the rat, at the end of the indifferent stage, the Mullerian duct is formed of tightly packed, wedge-shaped columnar cells with basal nuclei, many free ribosomes and a supranuclear Golgi apparatus (J.M. Price *et al.*, 1977).

Fallopian tube differentiation includes formation of fimbriae, folding in the ampullary region and acquisition of cilia and secretory capability by the epithelial cells. The fallopian tubes differentiate from the cranial portions of the Mullerian ducts and the caudal portions give rise to the uterovaginal canal (Figure 6.21). The uterotubal junction is demarcated by a dramatic increase in diameter of the uterine segment. The oviduct of the human and guinea pig is well developed at term, but in laboratory rodents tubal differ-

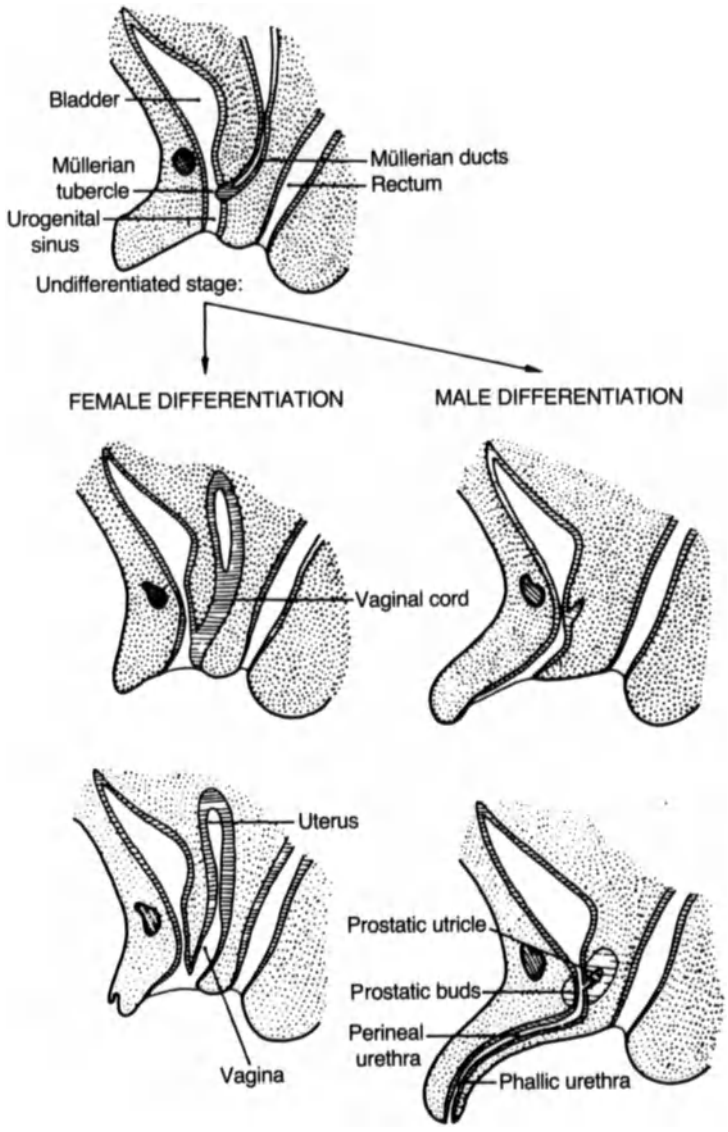

Figure 6.22 Differentiation of the urogenital sinus in male and female fetuses. In females, the vaginal cord develops between the urogenital sinus and the Mullerian ducts, and gains direct access to the perineum. In males, the utricular cord cavitates, to form the prostatic utricle, the male equivalent of the vagina, which opens between the vasa deferentia, just beneath the neck of the bladder. (Reproduced with permission from Josso, 1981.)

entiation is completed after birth (D. Price *et al.*, 1968).

Fusion of the Mullerian ducts to form the uterovaginal canal is complete by 66–68 GD in the human fetus (O'Rahilly, 1977). The cervix makes up the distal two-thirds of the fetal uterus with a slight curvature developing between the future cervical and fundic parts of the uterus at mid-gestation and the future ostium appearing by 100 GD (Bulmer, 1957). The endometrial and endocervical linings are derived from Mullerian duct epithelium and the adjacent mesenchyme contributes to the formation of the uterine muscular and connective tissue layers (Witschi, 1970). The basic structure of the uterus is established early in development. The cervical portion is larger than the body throughout fetal development. The proportions characteristic of the adult do not appear until the post-natal period. Mucin secretion in the endocervical canal occurs as early as 25 weeks of gestation and functional changes occur at this time resembling those elicited by oestrogen stimulation in the adult uterus.

The development of the vagina, and especially the origin of its epithelium, has been debated extensively in the embryological literature. There is still no general agreement regarding the relative contributions of these sources (O'Rahilly, 1977; J.G. Wilson, 1978). The current views are that the upper vagina and ectocervix develop from the Mullerian duct system as the caudal portion of the uterovaginal canal. The lower portion of the vagina is derived from the urogenital sinus.

During early development of the uterovaginal canal, the vaginal plate, a cord-like separation, forms between its lower portion and the cranial portion of the urogenital sinus (Figure 6.22). The hymen delineates the border between the vagina and urogenital sinus. By the fifth month of pregnancy, the fetal uterovaginal canal is patent and there is access to the exterior (O'Rahilly, 1977).

The epithelial lining of the vagina undergoes changes as it differentiates. During the time of vaginal plate formation, the cephalic portion is lined by a cuboidal epithelium. After differentiation of the vaginal plate, this epithelium, believed to be of Mullerian origin, degenerates and is replaced by a glycogen-rich, enzyme-poor, stratified squamous epithelium from the urogenital sinus, which joins with the columnar epithelium of the uterus just outside its external os (Bulmer, 1957; Forsberg, 1965; Gondos, 1985). This process begins late in the first trimester of pregnancy and is not completed until late in the fetal period. The dual origin of the vagina is exemplified by the abnormalities observed in patients with atresia of either Mullerian ducts or the distal vagina (Ulfedder and Robboy, 1976). There has been a renewed interest in the normal sequence of vaginal differentiation with the induction of vaginal abnormalities after prenatal exposure to diethylstilbestrol (A. Herbst *et al.*, 1971; Robboy *et al.*, 1977; R.F. Gasser, 1985).

(c) Urogenital sinus

The urogenital sinus is separated from the rectum when a transverse septum, the urorectal septum, divides the cloaca into the rectum dorsally and the urogenital sinus ventrally. The division of the cloaca begins in human embryos at 35–38 GD and is completed by 51–53 GD (Jirasek, 1971). After it is established, the urogenital sinus is divided into an upper pelvic portion and a lower phallic portion, which is in close association with the genital tubercle. The fused tips of the Mullerian ducts are separated from the dorsal wall of the urogenital sinus by a solid mass which may receive contributions from Mullerian, Wolffian and sinusal epithelium, although there is still considerable controversy regarding this point (Bulmer, 1957; Glenister, 1962; Forsberg, 1965; O'Rahilly, 1977).

In the male, the external genitalia arise in relation to the phallic portion of the definitive urogenital sinus (Gondos, 1985). The prostate

gland also develops in conjunction with the pelvic portion of the urogenital sinus with contributions from the primitive urethra. The prostate gland first appears as epithelial buds, extending laterally from the urogenital sinus near the Mullerian tubercle in human fetuses 61–63 GD (Figure 6.21) (Glenister, 1962). Mesenchymal differentiation precedes bud development, forming solid branching cords which develop a lumen followed by formation of tubules and alveoli. Ultrastructurally, the prostatic cells first resemble those of the stratified urethral epithelium. After lumen formation some apical cells become structurally polarized and the appearance of floccular material in the apical cytoplasmic granules indicates the onset of prostatic secretory activity (Kellokumpu-Lehtinen *et al.*, 1979).

Prostatic maturation in the rat is not far advanced at birth and most acini are still solid (Flickinger, 1970). The rat fetal prostatic and sinusal cells look similar and are characterized by confronting cisternae in the granular endoplasmic reticulum. Masculinization of the urogenital sinus involves regression and lack of descent of the vaginal anlage, closure of the urogenital folds, growth of the phallus and formation of the penile urethra (Figure 6.22).

In the female, the urogenital sinus gives rise to the lower two-thirds of the vagina, including the endodermal portion of the vaginal epithelium (Cunha, 1975; O'Rahilly, 1977). The urethral and the bulbourethral glands are also derived from the epithelium of the urogenital sinus. The lower part of the urogenital sinus forms the vestibule.

(d) External genitalia

At the indifferent stage of development the genital tubercle, a midline elevation, and the laterally located genital folds surround the urogenital membrane. The urogenital membrane ruptures, allowing the urogenital sinus to open onto the surface. An additional pair of swellings, the genital swellings, appear lateral to the genital folds (Figure 6.23).

Male

Lengthening of the anogenital distance marks the beginning of masculinization of the external genital organs in humans (Jirasek, 1971) and probably in other mammals as well. By the end of the first trimester the genital tubercle becomes elongated and is transformed into the cylindrical-shaped penis. The genital swellings develop into rounded scrotal folds which migrate caudally and then fuse below the base of the penis (Figure 6.23). The definitive urethral groove extends forward on the ventral aspect of the penis. The lining of the penile urethra is derived mainly from the endodermal portion of the urogenital sinus. The perineal raphe results from fusion of the urethral folds. The prepuce forms from a fold of skin at the base of the glans and the bulbourethral glands arise from the penile urethra as endodermal derivatives. The erectile tissue, the corpus spongiosum and the corpus cavernosa, develops within the mesenchymal core of the penis. Formation of the penis is completed by 100 GD in the human, but no appreciable difference exists between the penis and clitoris until the 16th gestational week (Feldman and Smith, 1975). Paradoxically, penile growth occurs mainly between 20 weeks' gestation and term, a time when serum testosterone levels are declining.

Female

The changes in the female are less extensive than in the male, and development of the external genitalia begins in the 8-week-old fetus. The genital tubercle does not continue to enlarge as in the male and is bent caudally, forming the clitoris. The lateral portions of the genital (labioscrotal) swellings enlarge to form the labia majora, and the urethral folds

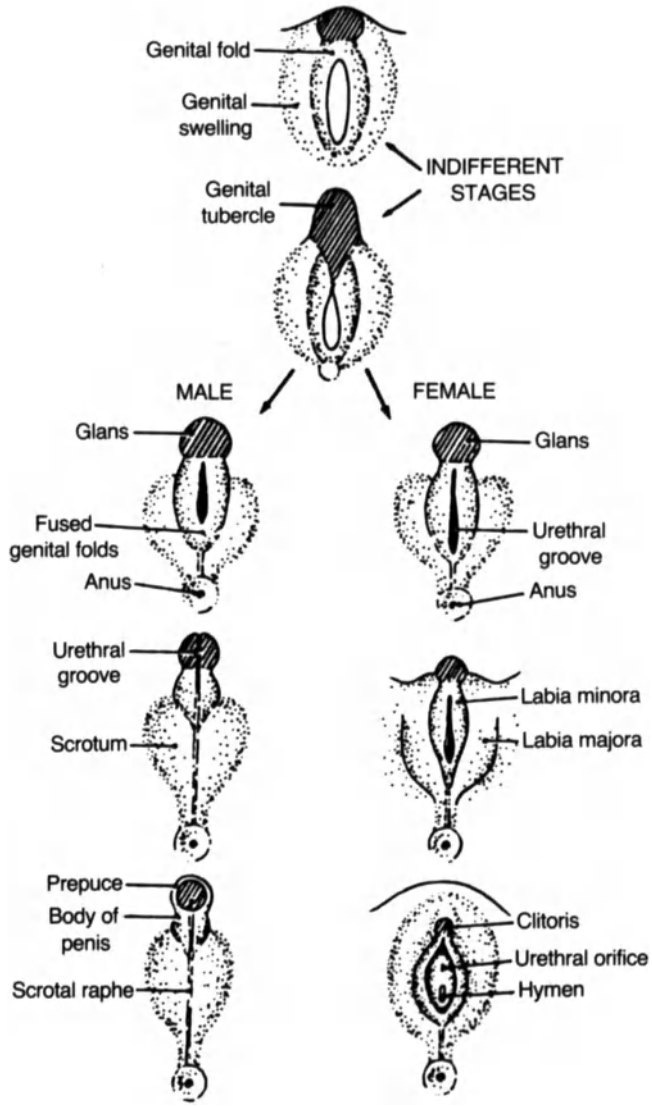

Figure 6.23 Differentiation of the external genitalia in males and females from identical primordia. (Reproduced with permission from J.D. Wilson *et al.*, 1981.)

which flank the urogenital orifice persist as the labia minora (Figure 6.23).

6.6.4 HORMONAL REGULATION

Jost (1947, 1953) was the first to elucidate the fundamental mechanism of sexual differentiation. No stimulation is known to be required for female differentiation while differentiation of the male genital tract is controlled by the fetal testis; in the absence of the fetal testis feminization occurs passively. Jost (1972) established that the castrated mammalian embryo of either sex develops as a

female, while male development is induced only in the presence of specific signals from the fetal testis.

The sexual differentiation of most mammals is thought to be hormonally mediated as a consequence of the genetic determination of gonadal sex. There is recent evidence that a marsupial mammal (*Macropus eugenii*) undergoes extensive sexual dimorphism preceding any form of gonadal morphological differentiation (Wai-Sum *et al.*, 1988). Thus, the conventional view that all somatic sexual dimorphisms in mammals are a consequence of gonadal hormone secretion requires further study, especially the effects of sex-linked genes (Jost and Magre, 1988).

(a) Role of testosterone

In the rabbit and human fetal testis, testosterone synthesis begins shortly after the onset of histological differentiation of the seminiferous tubules and at the time the Leydig cells appear (J.D. Wilson and Siiteri, 1973; Siiteri and Wilson, 1974). Testosterone, the main androgen produced by the male fetal testis (J.D. Wilson and Siiteri, 1973), has two known functions in the developing embryo. In the testis it has a local function promoting maturation of the seminiferous tubules. Systemically, after it is secreted into the fetal circulation, it has an essential function in Wolffian duct virilization and differentiation of the male genital tract. Five different genetic defects have been identified in man which illustrate the essential role of testosterone in the embryological development of the male genital tract (Bongiovanni, 1978; J.G. Wilson, 1978; Saenger, 1984). These defects are discussed later (section 6.7) under abnormal development of the genital tract.

There is considerable experimental evidence to demonstrate the role of the fetal testis in Wolffian duct development. If rabbit male fetuses are castrated, removing the source of testosterone, the Wolffian ducts regress unless testosterone is implanted (Jost, 1947). A similar response occurs *in vitro* in both rabbit and rats (Josso, 1970). Under similar circumstances female Wolffian ducts can be induced to persist, but only within their 'critical period' for sex differentiation.

In rabbits, rats and man the undifferentiated Wolffian ducts lack 5α-reductase, an enzyme which enables conversion of testicular testosterone into its 17-hydroxylated derivative, dihydrotestosterone (DHT) (J.D. Wilson and Lasnitzki, 1971; Siiteri and Wilson, 1974). After sexual differentiation, 5α-reductase levels rise as a consequence of androgen-mediated differentiation. The conversion of testosterone to dihydrotestosterone leads to masculinization of the urogenital sinus and external genital organs by means of DHT–androgen receptor protein interactions. Androgen receptors from fetal rat Wolffian ducts bind more readily with DHT than testosterone (Gupta and Block, 1976); this is also true of androgen receptors elsewhere in the body. In 14.5-day male and female rat fetuses the number of binding sites is similar but later increases in males. The presence of functional androgen receptors is now considered to be an absolute requirement for male sexual differentiation (Mowszowicz *et al.*, 1989).

At present there is considerable knowledge regarding the mechanisms of androgen action in the post-natal period. However, very little is known about what happens in the embryo because of its small size, which makes it difficult to study (J.D. Wilson *et al.*, 1981). Studies of single-gene mutations in animals and humans that cause resistance to androgen action and inhibition of male genital system development indicate that androgens act similarly during pre- and post-natal life (Griffin and Wilson, 1977, 1980).

(b) Role of Mullerian inhibiting substance

In addition to a stimulatory effect on the Wolffian duct system, the fetal testis has an inhibitory effect on Mullerian duct develop-

ment. This was first demonstrated in mammals by Jost (1947) in the rabbit fetus. He found that Mullerian ducts were refractory to high levels of testosterone and produced a specific inhibitor of the Mullerian duct which differed from testosterone. Since this initial critical observation, Picard *et al.* (1978) have demonstrated that the fetal testis contains an incompletely characterized glycoprotein with anti-Mullerian properties. This substance, or factor, is known as the anti-Mullerian hormone (AMH) or the Mullerian inhibiting factor and acts in the male to cause regression of the Mullerian ducts. AMH is formed by the Sertoli cells in the seminiferous tubules and is about 70 000 molecular weight (Blanchard and Josso, 1974; Donahoe *et al.*, 1977). Mullerian duct regression begins soon after differentiation of the seminiferous tubule. The formation of this inhibiting substance constitutes the first endocrine function of the embryonic testis (J.D. Wilson *et al.*, 1981). Although the mechanism by which AMH acts is unknown, recent evidence suggests that it may play a role in germ cell maturation (Hirobe *et al.*, 1992). Support for the concept that Mullerian regression is an active process comes from the human genetic disease (persistent Mullerian duct syndrome) in which genetic and phenotypic males have Fallopian tubes and a uterus, and the male Wolffian ducts are also present (Sloan and Walsh, 1976). This disease is inherited as a recessive trait, either autosomal or X-linked, and results from either a failure to produce AMH or an inability of the tissue to respond to the hormone. One of the main features of this disease is the failure of the testis to descend, particularly the transabdominal movement of the testis. This suggests that AMH plays an important role in testicular descent (J.D. Wilson *et al.*, 1981; J.D. Wilson, 1989).

(c) Role of oestrogens

The embryo is exposed to a wide array of hormones as it develops in the uterus. In contrast to androgens, the role of oestrogens, especially in the development of the genital system, is largely unknown. Oestrogen synthesis occurs in both male and female embryos prior to implantation, and this oestrogen may be essential to the process of implantation itself. Between 6 and 8 weeks of gestation both the ovaries and the testes of human embryos initiate characteristic endocrine function. Thus, the ovary acquires the capacity to secrete hormones at the same time as the testes (George and Wilson, 1978). If the synthesis or response to oestrogens is blocked, normal implantation may be prevented, resulting in early embryonic death. As yet no mutations have been characterized that result in either deficient oestrogen synthesis or unresponsiveness to its action (J.D. Wilson *et al.*, 1981). Later in pregnancy, oestradiol formation is initiated in the embryonic or early fetal ovary before its histological differentiation occurs. Oestrogen has also been shown to modify the Mullerian duct system experimentally, and oestrogen receptors have been demonstrated in Mullerian ducts of fetal rats and guinea pigs late in gestation. However, normal development occurs in castrated females, indicating that it is not an oestrogen-dependent process. The local action of oestradiol may play a role in the histological differentiation of the ovary, and exposure to diethylstilboestrol (DES), an exogenous oestrogen, alters sexual differentiation (McLachlan and Newbold, 1987). Aside from this, the role that oestrogen plays in the development of either sex is not yet known.

6.7 ABNORMAL DEVELOPMENT

6.7.1 MAJOR MALFORMATIONS OF THE REPRODUCTIVE SYSTEM

(a) Human pseudohermaphroditism

Female pseudohermaphroditism (FPH) is a form of phenotypic sex reversal. Human fe-

Table 6.12 Critical periods of organogenesis in animals (Reproduced with permission from Schardein, 1976)

Species	*Mean duration of gestation (days)*	*Critical period*[a] *(days)*
Hamster, golden	16	4–14
Mouse	19	7–16
Rat	21	9–17
Rabbit	31	8–21
Ferret	43	8–28
Cat	63	5–58; 5–15 (most favourable)
Dog	63	1–48; 8–20 (estimated)
Guinea pig	68	11–20
Pig	114	12–34
Sheep	150	14–36
Monkey, rhesus	168	20–45; 22–30 (most susceptible)
Monkey, baboon	175	22–47
Armadillo	225	1–30
Human	278	20–55
Cow	284	8–25
Horse	336	?

[a] Period of embryological organogenesis or period of known susceptibility to teratogens.

male pseudohermaphrodites are genetic, XX karyotype and gonadal females with normally developed but hypoplastic internal genitalia (Table 6.13).

The genitalia are ambiguous, i.e. male or female sex cannot be determined, or they are masculinized to a variable degree. A female fetus becomes masculinized if exposed to excessive androgens *in utero*. The androgens may be derived from the fetus itself, from a maternal source or from an external source operating via the mother (Polani, 1981a). The degree of masculinization is time dependent. If androgen exposure occurs before the 12th week of gestation the degree of masculinization may be so great that the urethral opening is penile. On the other hand, if androgen exposure occurs after the 12th week of gestation, when the vagina has already been separated from the urethra, only hypertrophy of the clitoris will occur (Saenger, 1984; Reindollar *et al.*, 1987). These individuals may be fertile and bear young once the source of the androgens has been removed.

Congenital adrenal hyperplasia is the most common cause of female pseudohermaphroditism, causing variable degrees of virilization of the external genitalia. Female pseudohermaphroditism is caused by three different forms of congenital adrenal hyperplasia, 3β-hydroxylated dehydrogenase deficiency, 2l-hydroxylase deficiency and 11-hydroxylase deficiency (New *et al.*, 1981). The external genitalia are normal in male fetuses exposed to excess testosterone from congenital adrenal hyperplasia. The female fetus with congenital adrenal hyperplasia is born with a normal uterus and Fallopian tubes because it does not possess a testis. Anti-Mullerian hormone is not produced. Thus, female genital abnormalities are present only in the external genitalia which is androgen responsive. The fact that the Wolffian duct undergoes complete involution in these cases

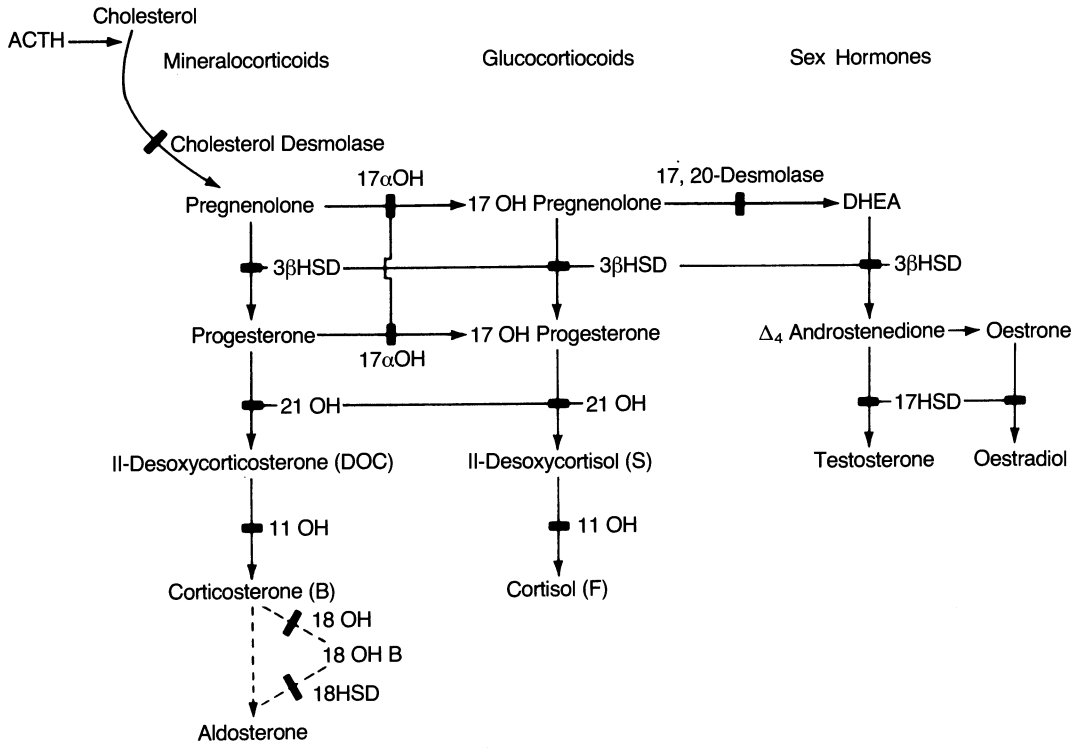

Figure 6.24 Synthesis of mineralocorticoids, glucocorticoids and sex steroids. (Reproduced with permission from Saenger, 1984.)

suggests that for normal Wolffian duct development the level of androgen is higher than that produced in congenital adrenal hyperplasia.

In cases of male pseudohermaphroditism (MPH) the individual with a 46XY karyotype has testes and possesses ambiguous external and internal genitalia or the genitalia resemble a female. Male pseudohermaphroditism can result from abnormalities in testosterone synthesis, testosterone metabolism or androgen action (Saenger, 1984; Reindollar *et al.*, 1987). Five distinct autosomal recessive disorders have been identified that result in reduced testosterone synthesis, caused by a deficiency in one of the five enzymes required for conversion of cholesterol to testosterone (Figure 6.24).

In three of these disorders, which involve deficiencies of cholesterol desmolase (Kirkland *et al.*, 1973; Bongiovanni, 1978), 3β-hydroxysteroid dehydrogenase (Bongiovanni, 1962; Schneider *et al.*, 1975; Pang *et al.*, 1983) and 17α-hydroxylase (New, 1970; Tourniaire *et al.*, 1976), cortisol as well as testosterone is deficient.

The clinical appearance of patients with these enzyme deficiencies is quite variable (C.R. King and Pernoll, 1984). The phenotypes range from the male with mild hypospadias to an apparent female with marked ambiguity. The Mullerian system is absent in these males because embryonic production of anti-Mullerian hormone is not affected by errors in androgen synthesis. The Wolffian system and the external genitalia are virilized to a variable degree.

In two of the disorders there are enzyme defects involving deficiencies of 17,20-desmolase (Zachmann *et al.*, 1972; Goebelsmann

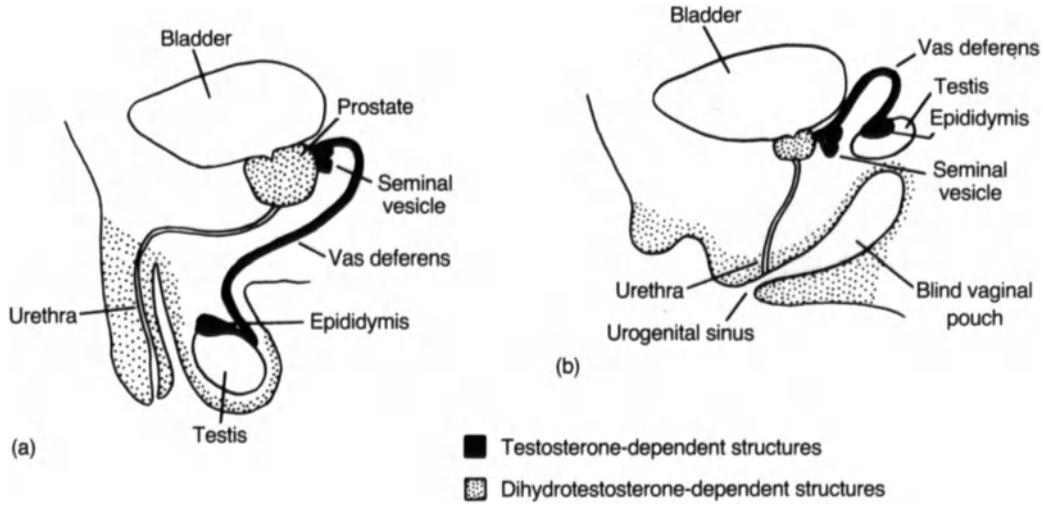

Figure 6.25 (A) Suggested selective roles of testosterone and dihydrotestosterone in normal male sexual differentiation. (B) Findings in males with inability to convert testosterone to dihydrotestosterone due to 5 α-reductase deficiency. Testosterone-dependent structures are normal, although testes are not descended and dihydrotestosterone-dependent structures are not developed. A blind vaginal pouch is frequently present. (Reproduced with permission from Saenger, 1984.)

et al., 1976; Zachmann *et al.*, 1982) and 17-hydroxysteroid dehydrogenase (Goebelsmann *et al.*, 1973; Givens *et al.*, 1974; Virdis *et al.*, 1978; Levine *et al.*, 1980) that primarily affect testosterone synthesis by the testes. Patients with 17-α-hydroxysteroid dehydrogenase deficiency are thought to be normal prior to puberty, however a blind-ending vaginal pouch without Mullerian derivatives is usually present (Goebelsmann *et al.*, 1973). Inguinal testes are usually present and gynaecomastia may develop from the peripheral conversion of weak androgens to oestrogens.

Male pseudohermaphroditism resulting from abnormal testosterone metabolism involves a deficiency of 5α-reductase and is the first inherited abnormality of steroid metabolism to be recognized in which the defect resides in target tissues (Imperato-McGinley *et al.*, 1974). The deficiency is an autosomal recessive defect, and causes abnormally low conversion of testosterone to DHT, the androgen responsible for masculinizing the external genitalia of the male fetus (Figure 6.25). Deficiency in 5α-reductase leads to pseudohermaphroditism in males who are born with ambiguous genitalia (Saenger, 1984). The clitoris is enlarged and often has a ventral urethral groove. A urogenital sinus is present and either a single orifice or separate urethral and vaginal orifices are present, depending upon the degree of masculinization. At puberty, the clitoris and the testes enlarge and the testes descend into the labioscrotal folds. A male phenotype develops, the voice deepens, and frequent erections occur. Because DHT does not play a role in female sexual differentiation or pubertal maturation homozygous females have a normal phenotype and are fertile (R.E. Peterson *et al.*, 1977). See Table 6.13.

(b) Animal pseudohermaphroditism

The spontaneous incidence of this condition has been reported only occasionally for animals, especially when compared with con-

Table 6.13 Classification of disorders (malformations) of sexual differentiation

Diagnosis	*Karyotype*	*External genitalia*	*Gonads*	*Internal ducts*	*Comments*
Female pseudohermaphroditism					
Congenital adrenal hyperplasia	46,XX	Ambiguous	Ovary	Mullerian	Female genital abnormalities are present only in the androgen-responsive external genitalia.
Maternal ingestion of androgens and synthetic progestins	46,XX	Ambiguous or male	Ovary	Mullerian	Exposure before 12 weeks causes formation of urogenital sinus; after that causes only clitoral enlargement
Male pseudohermaphroditism					
Abnormal testosterone synthesis	46,XY	Ambiguous, hypospadic male or female	Testis	Ambiguous or Wolffian	Enzyme defects affecting synthesis of testosterone and cortico-steroids (variants of congenital adrenal hyperplasia)
Abnormal testosterone metabolism	46,XY	Ambiguous	Testis	Wolffian	Defects in testosterone metabol-ism by peripheral tissues (5α-reductase deficiency).
True hermaphroditism	46,XX or 46,XY or mosaics	Variable but usually ambiguous	Testis + ovary or ovotestis	Mullerian + Wolffian	Cause is unclear; 46, XY karyotype; There is probably undetected chimerism or mosaicism and 46, XX sex chromosome mosaicism may occur
Testicular feminization	46,XY	Female	Testis	Wolffian	Phenotypic females because there is no response to testosterone or DHT because of receptor defect

ditions such as freemartinism, which are much more common (Benirschke, 1981). Both male and female pseudohermaphroditism have been observed in dogs (Phillips *et al.*, 1939; Fralick and Murray, 1948; Schultz, 1962; Curtis and Grant, 1964; McFeely and Biggers, 1965; Murti *et al.*, 1966; McFeely *et al.*, 1967) and are apparently more common in cocker spaniels. In 48 cases of canine intersex, 16 were found in cocker spaniels (Hare, 1976). Of these, nine were male pseudohermaphrodites with either scrotal or inguinal testes with ambiguous genitalia and 78XX karyotypes. Rieck (1973) reported on a female pseudohermaphroditic cow with atrophic ovaries. Bruere *et al.* (1969) identified a male pseudohermaphrodite with normal 54XY chromosomes. The external genitalia were female and testes were present in the ovarian positions but no uterus was present. Although intersex is common in goats (Koch, 1961) only a small number of pseudohermaphrodites have been reported (Basrur and Coubrough, 1964). The caprine male pseudohermaphrodite is an example of an animal in which testes develop in an XX individual lacking a Y chromosome (N.W. King, 1978). The presence of the homozygous autosomal dominant gene for polledness (absence of horns) in genetic female goats also results in male pseudohermaphroditism, or, sometimes, true hermaphroditism (Eaton, 1943; Soller *et al.*, 1969). The European roe deer has a relatively high incidence of both pseudo- and true hermaphroditism (Koch, 1961). Benirschke (1981) examined the autopsy reports of 3000 mammals from the San Diego Zoo and found only three intersex states. All three animals, a Nubian goat, a Himalayan goat and a Uganda kob, possessed characteristics of male pseudohermaphrodites with testes and external genitalia which were ambiguous or female.

(c) True hermaphroditism

This is a relatively rare condition in humans. Abnormal differentiation of both external and internal genitalia also occurs and the gonads are usually classified as ovary–testis or ovotestes–ovary (Van Niekirk, 1981). In the true hermaphrodite ovarian and testicular tissue coexist. Approximately 80% of individuals studied have 46XX karyotypes, 10% have 46XY and 10% have mosaicism. Assigning sex may be difficult except where ovarian and testicular tissues are aligned end-to-end; in these individuals differentiation is straightforward (Saenger, 1984; Reindollar *et al.*, 1987). In true hermaphrodites the fetal testis may inhibit Fallopian tube development but development of the uterus is not suppressed. The testicular tissue is defective in secreting androgens and the anti-Mullerian factor. This deficiency in testicular function leads to inadequate virilization of the external genitalia and the failure to inhibit Mullerian duct development.

The cause of true hermaphroditism is still unclear. In those cases with a 46XY karyotype, there may be chimerism or mosaicism which is undetected; in 46XX sex chromosome mosaicism, Y–X interchange, Y–autosome translocation and single-gene mutation are possible causes (Wachtel *et al.*, 1976). In patients with 46XX the immunological detection of H-Y antigen suggests that in many individuals X–Y interchange or a Y–autosome translocation may, in fact, take place. Additional support for this theory comes from two patients with a minute additional band on the short arm of one of the X chromosomes. The involvement of a Y–chromosome translocation that is too small for cytological detection may account for testicular differentiation in true hermaphrodites with a 46XX karyotype (Saenger *et al.*, 1976; Wachtel *et al.*, 1976).

Clinically, sex designation should be based on the appearance of the external genitalia (Saenger, 1984). With regard to surgical cor-

rection, in patients with an end-to-end arrangement of the ovary and testis, the ovarian tissue may be saved but in those individuals with an ovotestis the entire structure must be removed (Van Niekirk, 1974). Breast development and virilization may occur at puberty if the ovotestis is left in place. Menstruation occurs in two-thirds of the patients with a uterus (J.L. Simpson, 1976).

Only two true hermaphrodites have been reported in primates. Sullivan and Drobeck (1966) reported that the external and internal organs in a female rhesus monkey were normal except for the presence of bilateral typical ovotestes. The cortex of the ovaries contained primordial ova and the central portions contained closely packed tubules and some interstitial tissue. Sex chromatin-positive cells were also identified. Much earlier Harlan (1826) described a 2-year-old Bornean orang-utan, though his description of hair colour suggests that this animal was in fact a gibbon (Ruch, 1959). The penis had a hypospadic groove, and a vulva, vagina and a bifid scrotum were present. The testes were undescended, the epididymis was present but the seminal vesicles were absent. A normal uterus, uterine tubes and ovaries were present. Ruch (1959) states that this animal was mistakenly reported as hermaphrodite and was probably a normal female. The long pendulous nature of the clitoral skin in spider monkeys, a normal feature of the external genitalia in this species, is misleading and intersexuality is often erroneously suspected.

True hermaphrodites have been observed in cats but at a low incidence (Centerwall and Benirschke, 1973; Benirschke, 1981). In the dog, true hermaphrodites are more commonly reported but only a few cases have been documented chromosomally (Benirschke, 1981). True hermaphrodites have been reported in beagles, terriers, setters, cocker spaniels and in unspecified breeds (N.W. King and Garvin, 1964; Vandevelde, 1965; McFeely *et al.*, 1967; Gerneke *et al.*, 1968; Pullen, 1970; Selden *et al.*, 1978; Dain and Walker, 1979). The most common chromosomal complement observed in these studies was XX and a smaller number of XXY cells. True hermaphrodites are also rare in cattle (McFeely *et al.*, 1967; Dunn *et al.*, 1968, 1970) and great care must be taken to distinguish them from freemartins by employing genetic techniques.

True hermaphrodites have not been reported in horses but a variety of aneuploid states, mainly, the 63XO condition, is relatively common (Benirschke, 1981). A review by Giovanni and Cribiu (1978) on chromosomal and sex anomalies in horses included a total of 23 cases reported in the literature. True hermaphroditism, with ovotestis or unilateral testis as well as uteri and Barr bodies, has been reported in pigs (Johnston *et al.*, 1958). Basrur and Kanagawa (1971) found one of three true hermaphrodites to be an XX/XY whole-body chimaera. The two others had bilateral ovotestes, uterus and female external genitalia and were 38XX. Maik (1969) found XX lymphocytes in 25 intersex pigs, including many who were true hermaphrodites. True hermaphroditism among goats is uncommon (Koch, 1961). This is somewhat surprising since the frequency of genital defects is common (Benirschke, 1981). Genital anomalies in artiodactyla have been occasionally observed (Benirschke, 1981).

True hermaphrodites are uncommon in rats, hamsters and guinea pigs. In the rat at least one ovotestes was observed in those reported as true hermaphrodites (D.C. Johnson, 1966; Kikuchi *et al.*, 1977; N.W. King, 1978), and only one true hermaphrodite has been reported in the rabbit (Sheppard, 1943). A few spontaneous true hermaphrodites have been observed in other strains of mice (Lyon, 1969; N.W. King, 1978).

(d) Testicular feminization

Testicular feminization (TF) is a form of male pseudohermaphroditism in which the

gonads are testes and are frequently found in the inguinal canal, and the external genitalia are those of a normal female. In testicular feminization the karyotype is 46XY. Under the influence of the Y chromosome, the gonads differentiate as testes and secrete testosterone and dihydrotestosterone (DHT). The condition is rare, inherited and is determined by a recessive gene. There is no response to testosterone or DHT because of a receptor defect caused by a gene mutation on the X chromosome (Migeon *et al.*, 1981a; Brown and Migeon, 1986) and phenotypic females develop. In these female fetuses the testis secretes an anti-Mullerian hormone and there is a suppression in development of the Fallopian tubes, uterus, and upper third of the vagina (Migeon *et al.*, 1981b).

Testicular feminization may be detected at birth by the presence of inguinal hernia or after puberty by primary amenorrhoea. At puberty, the external genitalia are unambiguously female, the clitoris is normal in size and the breasts are normally developed. The vagina usually ends blindly, and may be shortened, depending on the relative contribution of the Mullerian ducts and the urogenital sinus to its formation (Migeon *et al.*, 1981b). Typical of prolonged cryptorchidism, spermatogenesis does not occur.

Androgen production in these cases is normal or may be higher than normal (Griffin and Wilson, 1980). The higher levels of androgen are probably secondary to elevated levels of luteinizing hormone, which may be due to a deficient feedback regulation caused by resistance to androgen at the hypothalamic–pituitary level. Higher levels of androgen result in the testes producing more oestrogen, which contributes to the appearance of normal female secondary sex characteristics at puberty. The resistance to androgen is caused by abnormalities of the androgen receptor. At least two genetic variants exist; the receptor-negative variant is characterized by undetectable DHT binding to the cytosol of fibroblasts and is caused by absence of the receptor protein or by changes in the structure of the receptor, resulting in a temperature-dependent loss of affinity for the steroid. The presence of unstable receptors has been observed in both complete and incomplete testicular feminization. The receptor-positive variant has both normal cytoplasmic and nuclear binding of DHT. It may represent a defect that is distal to the receptor, i.e. the generation site of specific messenger RNA in the nucleus (Amrhein *et al.*, 1976; Griffin, 1979). These two variants are important, especially in patients with incomplete testicular feminization. An infant with XY karyotype and inguinal testes and complete female external genitalia with undetectable androgen binding may be expected to feminize spontaneously at puberty. On the other hand, an infant with normal androgen binding may not be completely insensitive to androgens and may virilize at puberty (Saenger *et al.*, 1980).

Testicular feminization is less common in most animal species (Benirschke, 1981). Nes (1966) described a form of testicular feminization in cattle. Shaver (1967) described two female intersexes in rabbits with apparently normal XY karyotypes. They possessed testes containing spermatogonia but the seminiferous tubules lacked lumina. The Mullerian ducts were absent and the vaginas ended blindly, thus they can be interpreted to represent cases of testicular feminization. This syndrome has been characterized in the rat (Bardin *et al.*, 1970) and in the mouse (Lyon and Hawkes, 1970) with male pseudohermaphroditism. King-Holtzman rats also have features resembling testicular feminization with normal male karyotypes (Allison *et al.*, 1965). The pattern of inheritance in the rat is the same as that for man (Benirschke, 1981). It affects males only and is transferred as an X-linked recessive character or possibly as a sex-limited autosomal dominant trait (Stanley and Gumbrech, 1964). In these cases the scrotum does not form, the testes are in the inguinal position and spermatogonia are present.

The affected rats resemble some human cases of testicular feminization in that they show some resistance to androgen (Lyon *et al.*, 1981). In mice with testicular feminization the karyotype is XY and they are positive for H-Y (Lyon and Hawkes, 1970). The mice have blind vaginas, no uteri and the epididymides and vasa deferentia are small or absent. The testes, located in the inguinal region, contain spermatogonia and the trait is X linked.

Freemartinism, the most common form of sex differentiation altered by hormones, occurs most often in cattle and, much less commonly, in sheep, goats and pigs (Noden and deLahunta, 1985). The gonad of the sterile intersex female twinned to a normal male displays various forms of testicular organization and has an XX karyotype. This condition results from an anastomosis of the extraembryonic chorioallantoic blood vessels of the twins about a week before gonadal differentiation and allows for passage of fetal hormones and blood cells from one twin to another. Primary germ cells may also pass between twins and localize in the gonad but their fate is unknown. The gonadal sex reversal of freemartins is due to the passage of a substance promoting testicular development, presumably H-Y antigen, from the developing male twin to the female twin (Noden and deLahunta, 1985). Even though seminiferous tubules develop in the genetically female testes, spermatogenesis does not occur, probably because XX germ cells cannot form spermatocytes. Inhibition of the paramesonephric duct and differentiation of the mesonephric duct are variable. Male duct differentiation is most advanced in the region most proximal to the gonad as a result of production of androgens from the sex-reversed freemartin gonad. The external genitalia of the freemartin calf are predominantly female, although there is usually some evidence of virilization due to the circulating androgens. It is of interest to note that, even though twin marmoset monkey fetuses of opposite sex often share a common circulation as a result of placental blood vessel fusion, the normal development of the female fetus is unimpaired.

Both chimaeric and mosaic animals offer interesting opportunities for the study of more than one genotype within one organism. In a mosaic animal, because of mitotic non-disjunction or chromosomal loss, all genotypes originate from a single zygotic genotype. The expression of trisomy and the accompanying abnormalities depend on when during cleavage non-disjunction occurs and where the affected cells are located.

Chimaeric animals also have more than one genotype but they are derived from two or more zygotic genotypes and they can be produced by transplanting tissue or blastomeres from one animal to another. Chimaeric animals are valuable models for the study of placental function and maternal–fetal interactions. The most common naturally occurring chimaeras are believed to be due to fusion of zygotes with fertilized polar bodies or by the fusion of two cleavage-stage embryos (Noden and deLahunta, 1985). Numerous other disorders of sexual development occur in animals and man but they are beyond the scope of this book (Benirschke, 1981; Polani, 1981a,b).

6.7.2 EXPERIMENTAL TERATOLOGY

General conditions for the occurrence of malformations have become evident from the numerous studies that have been conducted in laboratory animals as experimental models or for the purpose of testing the safety of drugs and chemicals for human use or exposure. From these studies principles of the science of teratology have been formulated (J.G. Wilson, 1973; J.G. Wilson and Fraser, 1977; Persaud *et al.*, 1985; Schardein, 1985) which provide useful guidelines in determining susceptibility to teratogenesis. Susceptibility to teratogenesis is dependent upon an interplay of the following: species, stage of prenatal development, dosage, route

of administration and teratogenic mechanisms.

(a) Species

All species are not equally sensitive to teratogenesis by a given teratogen. Some of these species differences are due to genetic factors but such things as variability in litter response observed even in inbred animals is not attributed only to genetic factors. Observed species differences may also result from differences in the intrinsic sensitivities of the target tissues and pharmacokinetic differences that determine the crucial concentration–time relationships in the embryo. These include maternal absorption, distribution, protein binding, metabolism and excretion as well as placental transfer (Nau, 1987). Kalter (1968) reported that inter- and intra-species variability in teratogenic response may be manifested in the following ways:

1. An agent that is teratogenic in some species may have little or no teratogenic effect in others.
2. A teratogenic agent may produce similar defects in various species, but the defects will vary in frequency.
3. A teratogenic agent may induce certain defects in one species that are completely different from those induced in other species.

Numerous examples exist to amplify the point of inter- and intra-species variability. Man and higher primates are highly vulnerable to thalidomide, rabbits react in a somewhat similar fashion, but require a higher dosage; most other animals, including rats, are more resistant to thalidomide teratogenesis. Mice are generally susceptible to the induction of cleft palate by cortisone, hamsters and guinea pigs less so, and rats are usually refractory to this action. Aspirin at doses sufficient to induce cleft lip and other defects in mice have no effect in guinea pigs and monkeys (Trasler, 1965; Tanimura, 1972; Kromka and Hoar, 1973).

There are also genetic differences within given strains or breeds of the same species which influence teratogenesis. As an example, cortisone induced cleft palate 100% of the time in the A/J strain of mouse, while only 20% of the fetuses in the C57 B1/6J strain had cleft palate exposure to a similar dosing regimen (Kalter, 1968). Both the maternal and fetal genotypes play an important role in the response to cortisone through cleft palate induction. Other factors which are variables in strain susceptibility to teratogenic agents are maternal parity and weight, fetal weight, number of young, maternal basal metabolic rate, size and constitution of the placenta, intrauterine associations, fetal and maternal production of hormones and maternal utilization of vitamins and other essential substances (Schardein, 1976).

(b) Stage of prenatal development

It is generally accepted that drugs and chemicals must be administered during organogenesis in order to induce teratogenesis. The importance of this principle can be appreciated by the fact that in thalidomide teratogenesis it was the time of treatment rather than the dosage that was the decisive factor (J.G. Wilson, 1972). The organogenic period, the critical period for teratogenesis, varies among the species and is partly dependent upon the length of gestation (Table 6.12). It has been generally held that the conceptus is resistant to production of malformations during the predifferentiation period, i.e. the time from fertilization to the onset of organ differentiation (Figure 6.26).

However, there is growing evidence from studies in rodents that chemical exposure during preimplantation can cause subtle nonlethal mutations that result in shifts in the developmental programme of the embryo. The deleterious effects can range from intrauterine mortality to growth retardation

and frank malformations (see review by Iannaccone *et al.*, 1987). Additionally, exposure to developmental toxins (e.g. alcohol and hyperthermia) during the period of gastrulation has been associated with brain and facial anomalies in experimental animals and humans (Webster *et al.*, 1988).

The time of greatest teratogenic insult usually corresponds to the time when the embryo is undergoing marked morphological changes and a particular organ is developing most rapidly. In the human this occurs during the third and eighth weeks of gestation (Figure 6.27).

Ironically, the period of greatest susceptibility begins before pregnancy is usually diagnosed or is even suspected by many women. Following organogenesis, 9 weeks to term, the fetus becomes progressively less susceptible to teratogenesis. The most important

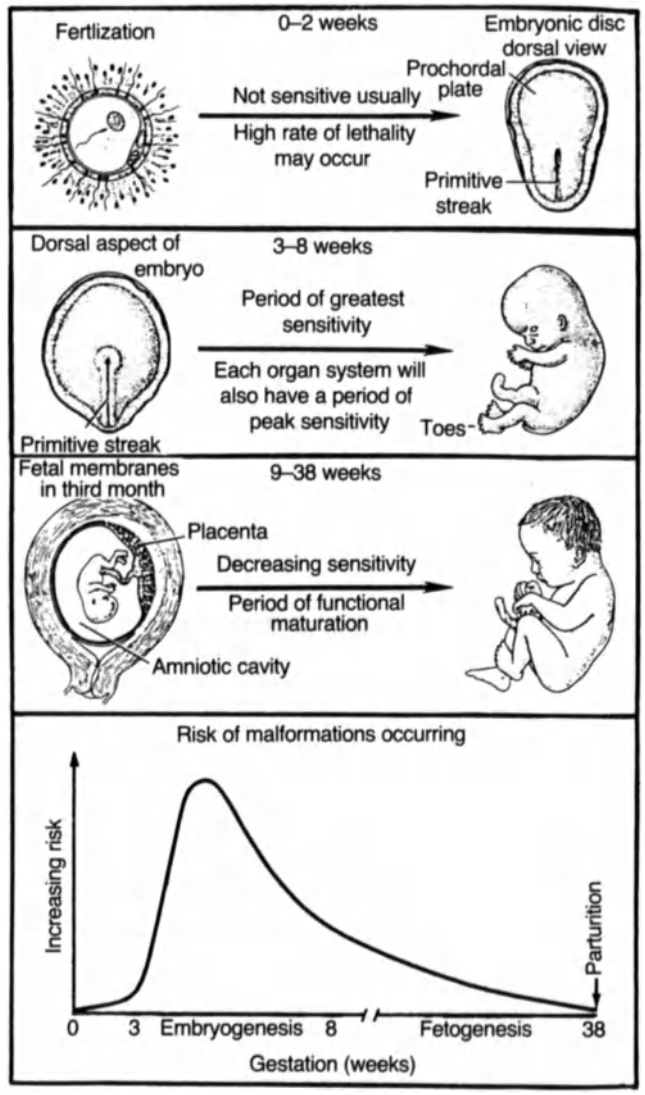

Figure 6.26 Periods of susceptibility to teratogenesis. (Reproduced with permission from Sadler, 1985.)

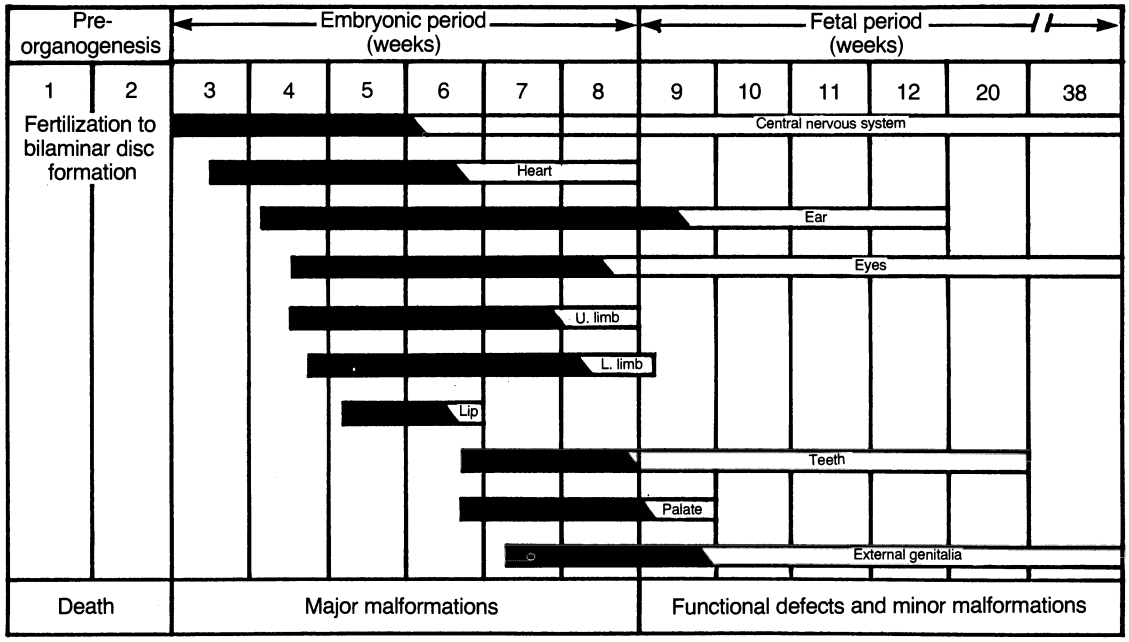

Figure 6.27 Susceptibility to teratogenesis for organ system (solid bar denotes highly sensitive periods). (Reproduced with permission from Sadler, 1985.)

features of the fetal period are marked histogenetic changes, rapid growth and functional maturation.

Administration of drugs that are dissolved, absorbed, metabolized and excreted rapidly can be confined to the period of organogenesis. This is the way most drugs are routinely tested. However, the activity of drugs that are absorbed or metabolized more slowly may occur outside the organogenic period and result in absence of a teratogenic effect. This factor explains why some drugs and chemicals administered outside (before or after) the critical period have shown teratogenic activity, and it also constitutes one of the common pitfalls in teratogenic testing.

(c) Dosage

The dosage of a potential teratogenic agent is very critical with regard to susceptibility to teratogenesis. In most instances the dosage of a teratogenic agent is within a narrow zone between that which will kill the fetus and that which has no discernible effect. It is only those doses that are sufficient to interfere with specific developmental events that are teratogenic, thus all doses are not teratogenic. The embryo is usually more susceptible to drugs than the adult; this is mainly because of the specific vulnerability of certain embryonic cells that is no longer present in the corresponding adult cells. Exposure of the embryo to greater concentrations of the drug than the adult is also possible. This may be due to the lack of development of the necessary enzymes in the embryo which are necessary for detoxification (Tuchmann-Duplessis, 1970).

There is considerable controversy concerning the relationship of embryolethality and malformation. Several authorities state that these two manifestations of abnormal development are different degrees of reaction to the same stimuli, with the rate of embryonic death and the rate and severity of malforma-

tions increasing in a somewhat parallel fashion as the dosage is increased (J.G. Wilson, 1959; Beck and Lloyd, 1963). These same authorities also state that the embryo has a threshold to dosage, above which irreparable alterations occur, resulting in malformation, or, secondarily, death. Other investigators claim that the correlation between embryolethality and malformation does not always exist, nor are these two manifestations of teratogenicity necessarily related (Tuchmann-Duplessis 1965, 1970; Gebhardt 1970; Kalter 1980; J.G. Wilson 1980).

In experimental settings, and probably in situations where accidental exposure to an environmental teratogen might occur, a suitable dosage of a teratogen usually results in the production of some normal offspring, some malformed offspring and some dead or resorbed offspring. Depending on the agent and the conditions of exposure, less common patterns of response may involve the production of intrauterine death in the absence of malformations (Neubert *et al.*, 1980) (see section 6.7.4, Teratogenicity of environemtnal agents) or vice versa. Experimentally, the selection of the proper dose level is, thus, of paramount importance. Teratogenicity, as in other toxicological studies, is governed by dose–response relationships. The response curve is usually quite steep and is of great significance in determining a true teratogenic effect. 'No effect' levels also have been shown for teratogens that have undergone realistic testing, however there is usually no relationship between teratogenicity and chemical structure or pharmacological properties of a drug.

(d) Route of administration

The route of administration of the teratogenic agent can influence the outcome of the experiment. For teratological testing it is usually recommended that the route (oral, parenteral, etc.) employed in human therapeutic use be used. The differences which occur in teratogenic response in many experimental studies are often due to the different routes of administration of the teratogenic agent. These responses are mostly related to differences in absorption and concentration of the drug, duration of exposure and speed of release, or to differences in metabolic fate and the nature of the metabolites reaching the embryo. As an example, hypervitaminosis A, a potent teratogenic procedure in animals, requires oily solutions of the vitamin to be effective orally.

To determine teratogenic mechanisms a number of unique methods of administering drugs have been employed. Turbow and Chamberlain (1968) used intra-amniotic injections to determine the direct effect of 6-aminonicotinamide on rat embryos. Wilk (1969) placed a millipore filter containing small amounts of chlorcyclizine directly on the amniotic sac or placenta of rats to demonstrate that the drug had a local action. W.J. Scott (1970) injected the diuretic drug acetazolamide into the yolk sac and/or decidua capsularis or into the exocoelom to produce the typical forelimb defect seen when given the drug orally.

6.7.3 TERATOGENICITY OF SEX STEROIDS

Little is known about the mechanisms of teratogenic action even though there is a wealth of information on the embryonic and fetal effects of drugs and chemicals in laboratory animals. J.G. Wilson (1973) has presented a sequence of metabolic and developmental events which may account for most abnormal development (Figure 6.28).

Hypothetically a sequence of molecular events occurs between the primary teratogenic insult and the final morphological defect; increased attention has been directed towards elucidating teratogenic mechanisms using a variety of experimental approaches, including *in vitro* culture and pharmacokinetics (McLachlan *et al.*, 1987, Welsch 1987). Beckman and Brent (1984) have summarized

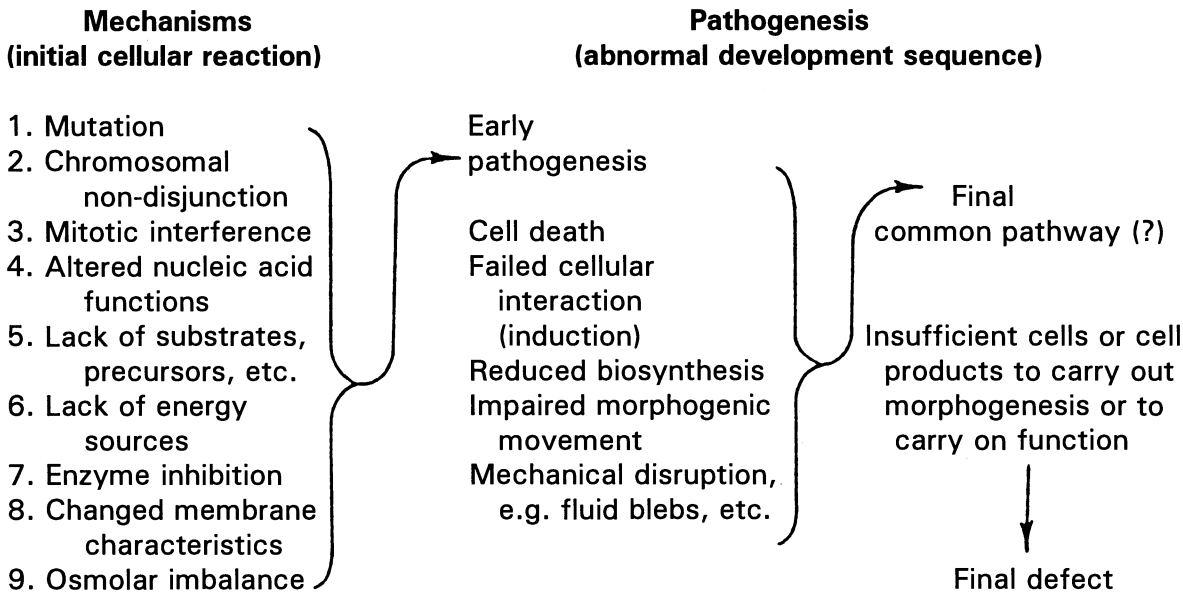

Figure 6.28 Schematic summary of initial cellular reactions and different types of pathogenesis converging into a final common pathway. (Reproduced with permission from J.G. Wilson, 1973.)

the mechanisms of action of known developmental toxins which may account for their embryopathy in humans.

Sex steroids

For the last several decades there has been much concern over the association between exogenous hormone therapy and a variety of birth defects. Worldwide clinical use of female sex hormones includes oral contraception, treatment for habitual or threatened abortion, management of dysmenorrhoea and dysfunctional uterine bleeding, antineoplastic therapy and, formerly, pregnancy testing.

Sex steroidal hormones, natural and synthetic, delivered singly or in combination, have been studied in several species of laboratory rodents, lagomorphs and non-human primates. Although there is a relatively wide range of embryotoxic effects reported for both the natural and synthetic oestrogens, there is no definable pattern; it appears that embryo or fetal death is common but teratogenicity very rare (Table 6.14).

These results reflect the known abortifacient effect of these compounds (McLean-Morris and van Wagenen, 1973; Neumann *et al.*, 1974). Genital tract abnormalities, including feminization of male fetuses and masculinization of female fetuses, have been reported in rodents exposed to some of these compounds during the period of sexual differentiation (Table 6.14).

Diethylstilboestrol (DES) has also been associated with genital tract anomalies in several test species. No one species precisely mimics the human response to *in utero* DES exposure which includes anatomical anomalies in the upper vagina, cervix and uterus, adenosis and clear cell adenocarcinoma in exposed females and oligospermia, variocoeles, epididymal cysts, cryptorchidism and testicular hypoplasia in exposed males (W.B. Gill, 1988; Rotmensch *et al.*, 1988). Similar reproductive anomalies in treated offspring of mice, rats, hamsters and rhesus monkeys have been observed (Khera, 1984; Walker, 1984; Hogan *et al.*, 1987).

While little or no teratogenicity has been reported in laboratory studies involving pre-

Table 6.14 Developmental toxicity of oestrogens in laboratory animals

Compound	*Species*	*Developmental toxicity*[a]	*Reference*
Oestradiol dipropionate	Mouse	GT	Raynaud (1958), Jean (1966)
	Mouse	NGT	Raynaud (1942)
	Mouse, rat	E, NGT	Jean and Jean (1970), Jean (1971)
	Rat	E, GT	Greene *et al.* (1940)
	Rhesus monkey	N	van Wagenen and Morse (1944), McLean-Morris and van Wagenen (1966)
Oestradiol 17-beta	Mouse	GT	Kimura (1975)
	Rat	E	Bodyazhina and Roganova (1966)
	Rabbit	E	McLean-Morris and van Wagenen (1956)
Oestradiol benzoate	Mouse	E, NGT	Nishihara (1958)
	Rat	E	Freire (1955)
	Rat	GT	Bengmark (1958)
Oestradiol valerate	Rat	E, NGT	Ornoy (1973)
Oestriol	Rhesus monkey	N	McLean-Morris and van Wagenen (1973)
Oestrone	Mouse	E, NGT	Nishihara (1958)
	Rat	E	Bodyazhina and Roganova (1966)
	Rhesus monkey	N	van Wagenen and Morse (1944)
Ethinyloestradiol	Rabbit	E	McLean-Morris and van Wagenen (1966)
Mestranol	Rabbit	E	Saunders and Elton (1967) McLean-Morris and van Wagenen (1966)
Diethylstilboestrol (DES)	Mouse	GT	Newbold and McLachlan (1982)
	Rat	GT	Vorherr *et al.* (1979)
	Guinea pig	N	Davies *et al.* (1985)
	Hamster	GT	T.M. Wilson *et al.* (1986)
	Rabbit	E	McLean-Morris and van Wagenen (1966)
	Rhesus monkey	GT	Hendrickx *et al.* (1979), L.D. Johnson (1984)
	Cebus monkey	GT	L.D. Johnson *et al.* (1981)

[a] N, no effect; E, embryolethality; GT, genital teratogenicity; NGT, non-genital teratogenicity.

natal administration of progesterone or its hydroxy analogue, the effects of *in utero* exposure to synthetic progestins include embryolethality, genital defects and/or non-specific malformations in non-genital organs (Table 6.15).

Persistence of Wolffian ducts, clitoral hypertrophy, increase in anogenital distance, fusion of labioscrotal folds and inhibition of normal vaginal, oviductal and ovarian development are among the defects that have been reported in masculinized female fetuses.

Table 6.15 Developmental toxicity of progestins in laboratory animals

Compound	*Species*	*Developmental toxicity*[a]	*Reference*
Progesterone	Mouse	N	Gabriel-Robez *et al.* (1972)
	Rat	N	Nalbandov (1958), Coyle *et al.* (1976)
		E	Piotrowski (1971), Bartholomeusz and Bruce (1976)
	Rabbit	GT	Piotrowski (1969, 1971)
	Rhesus monkey	N	Wharton and Scott (1964)
Hydroxyprogesterone caproate	Mouse	E	Seegmiller *et al.* (1983)
	Rat	N	H. Jung and Peters (1967)
	Rhesus monkey	N	Courtney and Valerio (1968)
	Rhesus and cynomolgus monkey	E	Hendrickx *et al.* (1987a)
Norethindrone	Rhesus monkey	E, GT	Wharton and Scott (1964)
	Rat	GT	Goldman and Bongiovanni (1967), Neumann *et al.* (1964)
Norethynodrel	Mouse	E, GT, NGT	Gidley *et al.* 1970
	Rat	N	Peterson and Edgren 1965
		E, GT	Roy & Kar 1967
Norethandrolone	Rat	E	Neuweiler and Richter (1964)
Norgestrel	Rat	E	Dasgupta *et al.* (1973)
Medroxyprogesterone acetate (MPA)	Mouse	N	Andrew and Staples (1977)
		E, NGT	Eibs *et al.* (1979)
	Rat	N	Andrew and Staples (1977)
			Bruce and Bartholomeusz (1976)
		GT	Suchowsky and Junkman (1961)
			Learner *et al.* (1962), Neumann *et al.* (1974)
	Rabbit	E, NGT	Andrew and Staples (1977)
		N	Bruce and Bartholomeusz (1976)
	Cynomolgus monkey	GT, NGT	Prahalada *et al.* (1985a)
	Baboon	GT, NGT	Prahalada *et al.* (1985b)
Chlormadinone acetate	Mouse, rabbit	E, NGT	Takano *et al.* (1966)
Ethynodiol diacetate	Rat	N	Saunders and Elton (1967)
	Rabbit	E	Saunders and Elton (1967)

[a] N, no effect; E, embrylethality; GT, genital teratogenicity; NGT, non-genital teratogenicity.

Table 6.16 Developmental toxicity of progestin–oestrogen combinations in laboratory animals

Compound	*Species*	*Developmental toxicity*[a]	*Reference*
Norethynodrel and mestranol	Mouse	N	Abbatiello and Scudder (1970)
Norgestrel and ethinyloestradiol	Rat	N	Edgren and Clancy (1968)
Ethynodiol diacetate and mestranol	Rat, rabbit	N	Saunders and Elton (1967)
Norethisterone and 1% mestranol	Mouse, rabbit	E	Takano *et al.* (1966)
Norethynodrel and 2% mestranol	Mouse	E	Takano *et al.* (1966)
Progesterone and oestradiol benzoate	Rat	E, NGT	Harkert and Von-Kreybig (1979), H. Jung and Peters (1967)
	Rhesus and cynomolgus monkey	E	Hendrickx *et al*, (1987b)
Hydroxyprogesterone caproate and oestradiol benzoate	Rat	E	H. Jung and Peters (1967)
Hydroxyprogesterone caproate and oestradiol valerate	Rhesus monkey	E	Hendrickx *et al.* (1987a)
Norethindrone acetate and ethinyloestradiol	Rhesus monkey	E	Prahalada and Hendrickx (1983)
	Rhesus monkey and baboon	E	Hendrickx *et al.* (1987b)
	Cynomolgus monkey	E, GT	Hendrickx *et al.* (1987b)

[a] N, no effect; E, embryolethality; GT, genital teratogenicity; NGT, non-genital teratogenicity.

Observed feminization effects include persistence of Mullerian ducts, hypospadias, cryptorchidism, testicular atrophy, arrested spermatogenesis and inhibition of prostate development in exposed males.

Studies using various sex steroid combinations have shown few or no teratogenic effects in non-genital organs and embryolethal effects only at the highest doses administered (Table 6.16).

Genital organ malformations (i.e. masculinization of female fetuses) have been observed at excessive doses of norethindrone and ethinyloestradiol administered to cynomolgus monkeys (Hendrickx *et al.*, 1987b).

In summary, the animal studies involving prenatal administration of natural or synthetic progestins and oestrogens alone, or in combination, have elicited embryolethality and malformations of the genital system with

no apparent effect on non-reproductive organs. The lack of clear association between exogenous hormone exposure during human pregnancy and non-genital teratogenicity (Schardein, 1980; J.G. Wilson and Brent, 1981; Wiseman and Dodds-Smith, 1984; Katz *et al.*, 1985) is supported by the animal data.

It is evident from the data presented above that a number of sex hormones (i.e. oestrogens, diethylstilboestrol and some synthetic progestins) interfere with normal genital system development. J.G. Wilson and Brent (1981) have attributed the alterations observed in both animal and human intersexes to the prenatal influence of hormones which are heterologous to the fetal genetic sex. Thus, intersexuality (masculinization or feminization) consists of partial or complete retention of duct derivatives and external genitalia characteristic of the opposite sex as well as some degree of suppression of normal reproductive organ development. This perturbation in the fetal hormone–target organ relationship is considered a specialized teratogenic response. Until recently, little consideration had been given to the effects of steroid hormones and brain development (McEwen, 1987). Rodent studies indicate that abnormal levels of systemic gonadal hormones during brain differentiation may lead to permanent functional and structural changes in the brain which are associated with reproduction dysfunction (Arai *et al.*, 1988). Future studies will expand our knowledge in this area, especially the action of environmental chemicals on brain development and behaviour.

Although the mechanism of hormonal-induced effects on prenatal sexual maturation is not well-understood, the possibilities include:

1. disruption of normal steroid synthesis and/or metabolism; and
2. interference with the normal steroid–receptor interaction.

Studies designed to explain the paradoxical effect of medroxyprogesterone acetate (MPA) on differentiating male and female external genitalia have shed some light on this topic. Three enzyme inhibition mechanisms have been proposed as possible mechanisms of MPA action:

1. inhibition of 3β-hydroxysteroid dehydrogenase, which results in an abnormal biosynthetic pathway leading to absence of androgen in male fetuses and production of weak androgens capable of virilizing the female fetus (Goldman and Bongiovanni, 1967);
2. blockage of 5α-reductase which is essential for conversion of testosterone to dihydrotestosterone (Voight and Hsia, 1973);
3. direct inhibitory action on testicular steroidogenesis (Rosenthal *et al.*, 1983).

That MPA might elicit its effects via interaction with receptors for sex steroids has also been proposed. Receptor studies in rats (Cupceancu and Neumann, 1969) indicate that MPA may have weak androgenic activity. This could result in the occupancy of androgen receptors and subsequent blocking of more potent endogenous androgens to produce an anti-androgenic (i.e. feminizing) effect in males. Conversely, a weak androgen receptor stimulation in females might be sufficient to produce virilization.

6.7.4 TERATOGENICITY OF ENVIRONMENTAL AGENTS

Drugs and other environmental chemicals have been studied in various animal species to determine their embryotoxic potential (see reviews by Hemminki and Vineis, 1985 and F.M. Sullivan and Barlow, 1985; Schardein and Keller, 1989). The focus of this section will be on a review of the data on embryotoxic effects of some of the environmental chemicals in animals and humans.

The toxic potential of metals is of special concern since these compounds may be bio-

Table 6.17 Developmental toxicity of metals in laboratory animals

Compound	*Species*	*Teratogenicity*[a]	*Developmental toxicity*[b]	*Reference*
Mercuric oxide	Rat	+	E,M	Rizzo and Furst (1972)
Mercuric acetate	Hamster	+	E,Gr,M	Gale and Ferm (1971)
Methylmercury hydroxide	Mouse	+	E,Gr,M	Su and Okita (1976)
Methylmercury chloride	Cat	+	E,M	Khera (1973)
Lead acetate	Hamster	+	M	Ferm and Carpenter (1967)
Lead nitrate	Hamster	+	M	Ferm and Carpenter (1967)
	Rat	+	M	McClain and Becker (1975)
Lead chloride	Hamster	+	M	Ferm and Carpenter (1967)
Tetraethyl lead	Rat	−	E,Gr	McClain and Becker (1972)
	Rat, mouse	−	E,Gr	Kennedy *et al.* (1975)
Tetramethyl lead	Rat	−	E,Gr	McClain and Becker (1972)
Trimethyl lead	Rat	−	E,Gr	McClain and Becker (1972)
Cadmium chloride	Rat	+	E,M	W. Chernoff (1973)
Chromium trioxide	Hamster	+	E,M	Gale (1978)
Manganese chloride	Hamster	−	E	Ferm (1972)
Nickel (carbonyl)	Rat	+	Gr,M	Sunderman *et al.* (1979)
Nickel (chloride)	Mouse	+	E,Gr,M	Lu *et al.* (1979)
Selenium (sodium selenite)	Mouse	−	Gr	Nobunaga *et al.* (1979)
Tellurium	Rat	+	M	Garrow and Pentschew (1964)

[a] Schardein (1985): +, teratogenic; −, non-teratogenic.
[b] E, embryolethality; Gr, growth retardation; M, malformation.

concentrated and enter the food chain whether they occur naturally or are released into the environment as industrial contaminants. To date, mercury is the only environmental metal that has been firmly established as being teratogenic in humans. This compound was responsible for 'fetal Minamata disease' in Japanese infants born to mothers exposed to mercury-containing fish between 1954 and 1960. Similar incidences of fetal neurological dysfunction associated with maternal ingestion of methylmercury have been reported in the USA and Iraq (J.G. Wilson and Fraser, 1977). The developmental toxicity of various mercuric compounds has been confirmed in laboratory studies using rats, hamsters, mice and cats (Table 6.17).

While lead has been determined teratogenic in laboratory animals, its primary developmentally toxic effect in humans is fetal wastage rather than malformations. The risks are less well defined following exposure to other heavy metals during human pregnancy (Schardein, 1985). Studies in laboratory animals have shown variable embryotoxic responses to miscellaneous metallic compounds following treatment during organogenesis (Table 6.17).

Much attention has been directed to the potential toxicity of the widely used and chemically diverse group of pesticides used to control unwanted insects, fungi and plants in the environment. Studies in laboratory animals testing the embryotoxicity of various fungicides, insecticides and herbicides have produced different effects on intrauterine

development which may be related to the highly variable experimental parameters including route, level and duration of dosage. A small number of these studies is summarized in Table 6.18 and the reader is referred to Schardein (1985) for a more complete listing.

The embryotoxic potential of many pesticides is speculative since adverse effects in laboratory animals have often been produced at or near maternal toxic levels and considerably above likely environmental levels (J.G. Wilson and Fraser, 1977).

Despite the widespread concern over the potential embryotoxicity of dioxin-contaminated 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following its use as a defoliant in Vietnam between 1961 and 1969, repeated attempts to validate the teratogenicity of this herbicide in humans in various parts of the world have not been successful (J.G. Wilson and Fraser, 1977; Schardein, 1985). This compound has been tested for teratogenic potential in multiple studies and the results have not been clear-cut. This may be due, in large part, to the quantity of dioxin (TCDD) present as a contaminant in the manufactured chemical which is thought to impart much of the toxicity of 2,4,5-T (Schardein, 1985).

Polybrominated and polychlorinated biphenyls (PBBs and PCBs) are regarded as potentially important environmental contaminants because of their extensive use as lubricants and heat transfer agents as well as ingredients of paints, varnishes and synthetic resins. Studies in mice, rats and non-human primates have resulted in varying degrees of embryotoxicity with the major effects manifested as embryo or fetal death and intrauterine growth retardation (Table 6.18). A clear association between these compounds and malformation rates in humans is yet to be established.

Carbon monoxide (CO) is a major environmental pollutant due to air pollution from industrial complexes and vehicular traffic as well as to cigarette smoking. The most com-

Table 6.18 Developmental toxicity of pesticides and pollutants in laboratory animals

Compound	*Species*	*Teratogenicity*[a]	*Developmental toxicity*[b]	*Reference*
Ethylene oxide	Mouse	+	E,M	Kimmel and LaBorde (1979)
	Rat	−	Gr	Snellings *et al.* (1979)
Tetrachlorophenol	Rat	−	N	Schwetz *et al.* (1974a)
Chlordecone	Mouse	−	E,M	Chernoff and Rogers (1976)
(Kepone)	Rat	+	E,Gr,M	N. Chernoff and Rogers (1976)
				Rosenstein *et al.* (1977)
Sodium arsenite	Mouse	+	E,Gr,M	Hood (1972)
Pentachlorophenol	Rat	+	E,M	Schwetz *et al.* (1974b)
Acrylonitrile	Rat	+	E,Gr,M	Murray *et al.* (1978a)
Polybrominated biphenyls	Mouse	+	Gr,M	Corbett *et al.* (1975)
	Rat	+	E,Gr,M	Beaudoin (1977)
Polychlorinated	Rat	−	E	Kato *et al.* (1972)
biphenyls	Primate	−	E,Gr	J.R. Allen and Barsotti (1976)
Carbon monoxide	Rabbit		N	Schwetz *et al.* (1979)
	Rabbit		E,Gr	Astrup *et al.* (1972)

[a] Schardein (1985): +, teratogenic; −, non-teratogenic.
[b] E, embryolethality; Gr, growth retardation; M, malformation; N, no effect.

Table 6.19 Developmental toxicity of plastics and solvents in laboratory animals

Compound	*Species*	*Teratogenicity*[a]	*Developmental toxicity*[b]	*Reference*
Styrene	Mouse, hamster	−	E	Kankaanpaa *et al.* (1980)
	Rat	−	E,Gr	Ragule (1974) Murray *et al.* (1978b)
	Rabbit	−	Gr	Murray *et al.* (1979a)
Vinylidene chloride	Mouse	−	E,Gr	R.D. Short *et al.* (1976)
	Rat	−	E,Gr,M	R.D. Short *et al.* (1976)
	Rat	−	Gr	Murray *et al.* (1979b)
	Rabbit	−	E,Gr	Murray *et al.* (1979b)
Acrylic acid	Rat	+	M	A.R. Singh *et al.* (1972a)
Methacrylates	Rat	+	E,M	A.R. Singh *et al.* (1972a)
Phthalates	Rat	+	E,M	A.R. Singh *et al.* (1972b)
	Rabbit	−	N	J.A. Thomas *et al.* (1979)
Benzene	Mouse	+/−	M	Watanabe *et al.* (1968)
	Mouse	+/−	Gr	Murray *et al.* (1979b)
Carbon disulphide	Rat	+	E,Gr,M	Tabacova *et al.* (1978)
Methyl ethyl ketone	Rat	+	Gr,M	Schwetz *et al.* (1974c)
Toluene	Mouse	+	E,Gr,M	Nawrot and Staples (1979)
	Rat	−	E,Gr,M	Hudak *et al.* (1977)
Ethylene glycol	Rat, mouse	+	E,Gr,M	C.J. Price *et al* (1985)

[a] Schardein (1985): +, teratogenic; +/−, equivocally teratogenic; −, teratogenic.
[b] E, embryolethality; Gr, growth retardation; M, malformation; N, no effect.

mon embryotoxic effects that have been associated with CO poisoning during pregnancy are fetal death and adverse neurological signs in surviving offspring. Studies in laboratory animals have not shown consistent teratogenicity of this environmental agent (Table 6.18).

The possible embryotoxicity of solvents is of concern because of their widespread industrial and household use as well as their generally high rates of absorption via the skin, lungs and digestive tract. Although associations have been made between several industrial solvents, particularly benzene and chlorinated hydrocarbons, and birth defects, the data are too inconsistent to draw definitive conclusions regarding the teratogenicity of these compounds. However, the developmental toxicity of several solvents tested in laboratory animals (Table 6.19) is suggestive of their embryotoxic potential in humans. Most of the animal studies conducted testing agents used in the production of plastics (e.g. styrene and vinylidene chloride) have not verified their embryotoxicity (Table 6.19).

Careful analysis of the human data has not yet indicated a causal association of these agents with birth defects (Schardein, 1985).

In summary, the value of animal teratogenicity testing to reduce human exposure to hazardous agents during pregnancy is justified by the fact that all human teratogens also induce some form of developmental toxicity in at least one test species. The protocols originally established by the US Food and Drug Administration (FDA, 1966) and the World Health Organization (WHO, 1975) recommend two test species; the rat and the rabbit are most commonly used, although the mouse is often a substitute for either of these species. The role of the non-human primate in teratological research is particularly valu-

able where equivocal results are experienced in these commonly used laboratory species, when the drug/chemical is likely to be used during pregnancy and for human-derived biotechnical products which often are not bioactive in non-primate species (Hendrickx and Binkerd, 1990).

Although the practical aspects of current testing procedures do not require alteration, incorporation of pharmacokinetic and metabolism data is strongly advised in order to account for species differences in teratogenic susceptibility (N.A. Brown and Fabro, 1983). This information provides quantitative estimates of hazard in teratogenicity tests and facilitates risk extrapolation. Further, the evaluation of all manifestations of abnormal development in animal studies, i.e. malformations, embryolethality, growth retardation and functional alteration, is essential due to species-specific responses to prenatal insults (Schardein and Keller, 1989). The guidelines recently published by the US Environmental Protection Agency (1986) clearly address these four end-points in the assessment of developmental toxicity. The incorporation of maternal toxicity evaluation in test protocols has also received increased support, particularly with regard to the risk assessment process (Kimmel *et al.*, 1986). Thus, multiple factors must be taken into consideration when extrapolating the evidence of animal studies to the human situation.

6.8 CONCLUDING COMMENTS

In this chapter we have covered the maternal recognition of pregnancy during embryo implantation in primates, together with the regulation of the corpus luteum to the extent currently understood in primates. We then explored the normal and abnormal embryo fetal development in mammals, giving emphasis to primate systems, including the rates of development and the factors inducing abnormal differentiation. These factors may be genetically derived; may result from disorders at fertilization or synchronization of the embryo maternal dialogue during early pregnancy; or may be the consequences of teratogenic agents introduced inadvertently from therapy or environmental hazards. An experimental approach to understanding the effects of teratogens can help us to avoid their use and can also give us new probes that clarify the regulation of embronic and fetal development.

The term reproductive health normally covers the treatment of infertility and the regulation of fertility. In the broader context it covers also the maturation of gametes and the well-being of the embryo and fetus to birth together with the understanding of how these stages are perturbed by both intrinsic and extrinsic factors.

Our basic knowledge in this field requires considerable extension at present as a whole new dimension is opening up. The regulatory mechanisms of reproduction at molecular and cellular levels in real embryo–fetal time can now be approached. The new productive technologies, which include *in vitro* maturation of oocytes (R.M. Moore and Trounson, 1977), *in vitro* fertilization (Edwards, 1980), storage of games and embryos from a range of species by cryopreservation (Whittingham, 1971; Wilmut, 1972; Summers *et al.*, 1987), embryo transfer to the same or closely related species (Hearn and Summers, 1986) and the manipulation of embryos to produce twins, clonal products, transgenic cells or individuals through microinjection and stem cell transfer, open up new prospects for significant improvements to the reproductive health of animals and man.

The work of Surani (1986) and others is now opening up a greater appreciation of the interplay between maternal, paternal and embryonic genomes as well as the regulation of totipotency and pluripotency in embryos through a definition of cell lineage and the role of stem cells in rodents (Johnson *et al.*, 1986). At present we are still at the early stages of extending this knowledge to pri-

mate systems. Oocyte development and fertilization *in vitro* was reviewed by Boatman (1987), and there is now a range of species in which embryo culture, storage, transfer and successful development to birth have been achieved, for example the cynomolgus monkey (Balmaceda *et al.*, 1986), the baboon (Pope *et al.*, 1984), the rhesus monkey (Bavister, 1987) and the marmoset money (Summers *et al.*, 1987). Non-human primate systems will be essential for the future development of gene therapy or the cloning and accelerated production of new models for the disorders of early pregnancy.

More recently, our understanding of the genetic regulation of the maternal to embryo transition, the transcription, expression and secretion of embryo signals and of differentiation signals is being clarified. The prospects for gene injection, the repair of genetic deficiency and the precise isolation and transfer of genes are now real. The development of new cell and whole-animal analogues for human disease requires markers in *in vivo* and *in vitro* systems to increase the efficiency of monitoring and screening both the successful integration of genes and the physiological consequences of such manipulation.

Among the applications that will flow from such fundamental knowledge are novel diagnostics in molecular medicine, new developments in agriculture and animal science, including cloning of livestock (Fehilly *et al.*, 1984; reviewed by Polge, 1985), and the potential for industrial exploitation in high-value enzymes, reagents and biotechnological systems.

Looking to the future, a greater efficiency in our exploitation of the regulatory mechanisms of embryo–fetal development promises improved health and a greater choice in fertility regulation. The next 50 years will see a doubling of the human population from 5 to 10 billion, raising fundamental ethical and practical questions about genetic diversity and species survival. A balance in human and animal populations, with greater expectation of health, of which reproductive health is an important part, will be a vital need in achieving a solution.

REFERENCES

Abbatiello, E. and Scudder, C.L. (1970) The effect of Norethynodrel with Mestranol treatment of pregnant mice on the isolation-induced aggression of their male offspring. *International Journal of Fertility*, **15**, 182–189.

Abbott, D.H. and Hearn, J.P. (1979) The effects of neonatal exposure to testosterone on the development of behaviour in female marmoset monkeys in *Sex, Hormones and Behavior*. Ciba Foundation Symposium 62 (new series), Excerpta Medica, New York, pp. 299–316.

Abel, E.L. and Dintcheff, B.A. (1978) Effects of prenatal alcohol exposure on growth and development in rats. *Journal of Pharmacology and Experimental Therapeutics*, **207**, 916–921.

Abramovich, D.R. (1969) The weight of the placenta and membrane in early pregnancy. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **76**, 523–526.

Adams, C.E. (1960) Prenatal mortality in the rabbit, *Oryets lagus cuniculus*. *Journal of Reproduction and Fertility*, **1**, 36–44.

Adams, C.E. (1970) Ageing and reproduction in the female mammal with particular reference to the rabbit. *Journal of Reproduction and Fertility* Suppl. 12, 1–16.

Agelopoulou, R., Magre, S., Patsavoudi, E. and Jost, A. (1984) Initial phases of the rat testis differentiation *in vitro*. *Journal of Embryology and Experimental Morphology*, **83**, 15–31.

Alila, H.W., Dowd, J.P., Corradino, R.A., Harris W.V. and Hansel, W. (1988) Control of progesterone production in small and large bovine luteal cells separated by flow cytometry. *Journal of Reproduction and Fertility*, **82**, 645–665.

Allen, L.S., Hines, M., Shryne, J.E. and Gorski, R.A. (1989) Two sexually dimorphic cell groups in the human brain. *Journal of Neuroscience*, **9**, 497–506.

Allen, J.R. and Barsotti, D.A. (1976) The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. *Toxicology*, **6**, 331–340.

Allison, J.E., Stanley, A.J. and Gumbreck, L.G. (1965) Sex chromatin and idiograms from rats exhibiting anomalies of the reproductive organs. *Anatomical Record*, **153**, 85–92.

Alliston, C.W., Howarth Jr., B. and Ulberg, L.C. (1965) Embryonic mortality following culture *in vitro* of one- and two-cell rabbit eggs at elevated temperatures. *Journal of Reproduction and Fertility*, **49**, 337–341.

Amrhein, J.A., Meyer, W.J.III, Jones Jr., H.W. and Miegon, C.J. (1976) Androgen insensitivity in man: Evidence for genetic heterogeneity. *Proceedings of the National Academy of Sciences of the USA*, **73**, 891–894.

Anderson, D.H. (1927) The rate of passage of the mammalian ovum through various portions of the fallopian tube. *American Journal of Physiology*, **82**, 557–569.

Anderson, G.B. (1977) Fertilization, early development, and embryo transfer in *Reproduction in Domestic Animals*, 3rd edn (eds H.H. Cole and J.P. Cupps), Academic Press, New York, pp. 285–314.

Andrew, F.D. and Staples, R.E. (1977) Prenatal toxicity of Medroxyprogesterone acetate in rabbits, rats and mice. *Teratology*, **15**, 25–32.

Andrews, E.J. (1974) Pulmonary pathology in stillborn nonhuman primates. *Journal of American Veterinary Medical Association*, **164**, 715–718.

Angulo, Y. and Gonzalez, A.W. (1932) The prenatal growth of the albino rat. *Anatomical Record*, **52**, 117–138.

Arai, Y., Matsumoto, A., Yamanouchi, K. and Nishizuka, M. (1988) Perinatal sex steroid exposure, brain morphology, and neuroendocrine and behavioral functions in *Toxicity of Hormones in Perinatal Life*, (eds T. Mori and H. Nagasawa), CRC Press, Boca Raton, Florida, pp. 9–20.

Arbab-Zadeh, V.A. (1965) Tierversuche mit thalidomid und thalidomid-serum-mischung. *Medizinische Klinik*, **60**, 1733–1736.

Aref, I. and Hafez, E.S.E. (1973) Utero-oviductal motility with emphasis on ova transport. *Obstetrics and Gynecology Survey*, **28**, 679–703.

Arey, L.B. (1974) *Developmental Anatomy*, 7th edn, W.B. Saunders, Philadelphia.

Arnold, A.P. and Gorski, R.A. (1984) Gonadal steroid induction of structural sex differences in the central nervous system. *Annual Review of Neuroscience*, **7**, 413–442.

Astrup, P., Trolle, D., Olsen, H.M. and Kjeldsen, K. (1972) Effect of moderate carbon-monoxide exposure on fetal development. *Lancet*, **2**, 1220–1222.

Atkinson, L.E., Hotchkiss, J., Frtiz, G.R., Surve, A.H., Neill, H.D. and Knobil, E. (1975) Circulating levels of steroids and chorionic gonadotropin during pregnancy in the rhesus monkey, with special attention to the rescue of the corpus luteum in early pregnancy. *Biology of Reproduction*, **12**, 335–345.

Auletta, F.J. and Flint, A.P.F. (1988) Mechanisms controlling corpus luteum function in sheep, cows, nonhuman primates and women especially in relation to the time of luteolysis. *Endocrine Reviews*, **9**, 88–105.

Auletta, F.J., Agins, H. and Scommegna, A. (1978) Prostaglandin F mediation of the inhibitory effect of estrogen on the corpus luteum of the rhesus monkey. *Endocrinology*, **103**, 1183–1189.

Auletta, F.J., Kamps, D.L., Pories, S., Bisset, J. and Gibson, M. (1984a) An intra-corpus luteum site for the luteolytic action of prostaglandin $F_{2\alpha}$ in the rhesus monkey. *Prostaglandins*, **27**, 285–298.

Auletta, F.J., Paradis, D., Wesley, M. and Duby, R.T. (1984b) Oxytocin is luteolytic in the rhesus monkey (*Macaca mulatta*). *Journal of Reproduction and Fertility*, **72**, 401–406.

Auletta, F.J., Paradis, D.K. and Gibson, M. (1985) Luteolysis in the rhesus monkey: effect of chronic intra–corpus luteum infusion of estrogens on luteal function (abstract). *Fertility and Sterility*, pp.127:137.

Auletta, F.J., Jones, D.S.C. and Flint, A.P.F. (1988) Does the human corpus luteum synthesize neurohypophysial hormones? *Journal of Endocrinology*, **116**, 163–165.

Austin, C. R., Edwards, R.G. and Mittwoch, U. (1981) Introduction, in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 1–54.

Ayoub, D.M., Greenough, W.T. and Juraska, J.M. (1983) Sex differences in dendritic structure in the preoptic area of the juvenile macaque monkey brain. *Science*, **219**, 197–198.

Baird, A. and Walicke, P.A. (1989) Fibroblast growth factors. *British Medical Bulletin*, **45**, 438–452.

Baird, D.T. (1985) Control of luteolysis in *The Luteal Phase* (ed. J.J. Jeffcoate), John Wiley & Sons, Chichester, pp. 25–43.

Baird, D.T., Baker, T.G., McNatty, K.P. and Neal, P. (1975) Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. *Journal of Reproduction and Fertility*, **45**, 611–619.

Baker, L.N., Chapman, A.B., Grummer, L.N. and Casida, L.E. (1958) Some factors affecting litter

size and fetal weight in purebred and reciprocal-cross matings of Chester White and Poland China swine. *Journal of Animal Science*, **17**, 612–621.

Baker, T.G. (1963) A quantitative and cytological study of germ cells in human ovaries. *Proceedings of the Royal Society of London (Biology)*, **158**, 417–433.

Balmaceda, J.P., Heltman, T.O., Garcia, M.R., Paverstine, C.J. and Pool, T.B. (1986) Embryo cryopreservation in cynomologous monkeys. *Fertility and Sterility*, **45**, 403–406.

Bardin, C.W., Bullock, L., Schneider, G., Allison, J.E. and Stanley, A.J. (1970) Pseudohermaphrodite rat: End organ insensitivity to testosterone. *Science*, **167**, 1136–1137.

Barron, S.L. and Vessey, M.P. (1966) Birthweight of infants born to immigrant women. *British Journal of Preventative and Social Medicine*, **20**, 127–134.

Basrur, P.K. and Coubrough, R.I. (1964) Anatomical and cytological sex of a Saanen goat. *Cytogenetics*, **3**, 414–426.

Basrur, P.K. and Kanagawa, H. (1971) Sex anomalies in pigs. *Journal of Reproduction and Fertility*, **26**, 369–371.

Bavister, B.D. (1987) Oocyte maturation and *in vitro* fertilisation in the rhesus monkey in *The Primate Ovary* (ed. R.L. Stouffer), Plenum Press, London, pp. 119–137.

Bavister, B.D. and Boatman, D.E. (1989) *In vitro* fertilisation and embryo transfer technology as an aid to conservation of endangered species. *Zoo Biology* Suppl. 1, 21–31.

Bazer, F.W. and First, W.L. (1983) Pregnancy and parturition. *Journal of Animal Science*, **57**, 425–460.

Bazer, F.W., Sharp, D.C., Thatcher, W.W. and Roberts R.M. (1981) Comparative approach to mechanisms in the maintenance of early pregnancy in *Reproductive Processes and Contraception*, (ed. K.W. McKerns), Plenum Press, New York, pp. 581–618.

Bazer, F.W., Geisert, R.D., Thatcher, W.W. and Roberts, R.M. (1982) The establishment and maintenance of pregnancy in *Control of Pig Reproduction* (eds J.A. Cole and G.R. Foxcroft), Butterworth Scientific, London, pp. 227–252.

Bazer, F.W., Vallet, J.L., Roberts, R.M., Sharp, D.C. and Thatcher, W.W. (1986) Role of conceptus secretory products in establishment of pregnancy. *Journal of Reproduction and Fertility*, **76**, 841–850.

Beach, F.A., Kuehn, R.E., Sprague, R.M. and Anisko, J.J. (1972) Coital behavior in dogs. XI. Effects of androgenic stimulation during development on masculine mating responses in females. *Hormones and Behavior*, **3**, 143–168.

Beal, W.E., Lukaszewska, J.H. and Hansel, W. (1981) Luteotrophic effects of bovine blastocysts. *Journal of Animal Science*, **52**, 567–574.

Beaudoin, A.R. (1977) Teratogenicity of polybrominated biphenyls in rats. *Environmental Research*, **14**, 81–86.

Beck, F. (1978) Ferrets (developmental abnormalities) in *Pathology of Laboratory Animals* (eds K. Benirschke, F.M. Garner and T.C. Jones), Springer-Verlag, New York, pp. 1869–1873.

Beck, F. and Lloyd, J.B. (1963) An investigation of the relationship between foetal death and foetal malformation. *Journal of Anatomy*, **97**, 555–564.

Beckman, D.A. and Brent, R.L. (1984) Mechanisms of Teratogenesis. *Annual Review of Pharmacology and Toxicology*, **24**, 483–500.

Beer, A.E., Quebbeman, J.F., Ayers, J.W.T. and Haines, R.F. (1981) Major histocompatibility complex antigens, maternal and paternal immune responses, and chronic habitual abortion in humans. *American Journal of Obstetrics and Gynecology*, **141**, 987–999.

Begley, D.J., Firth, J.A. and Hoult, J.R.S. (1980) *Reproduction and Developmental Biology*, The Macmillan Press Ltd, London.

Behrman, H.R. and Hichens, M. (1976) Rapid block of gonadotrophin uptake by corpora lutea *in vivo* induced by prostaglandin $F_{2\alpha}$. *Prostaglandins*, **12**, 83–95.

Beling, C.G., Marcus, S.L. and Markham, S.M. (1970) Functional activity of the corpus luteum following hysterectomy. *Journal of Clinical Endocrinology*, **30**, 30–39.

Bendz, A., Einer-Jensen, N., Lundgren, O. and Janson, P.O. (1979) Exchange of krypton-85 between blood vessels of the uterine adnexa. *Journal of Reproduction and Fertility*, **57**, 137–142.

Bengmark, S. (1958) Influence of sex hormones on the embryonic development of the male genital organs, in *The Prostatic Uretha and Prostatic Glands*, (ed. S. Bengmark), CWK Gleerup, Lund Berlingska Doktryckeriet, Lund, pp. 91–128.

Benirschke, K. (1981) Hermaphrodites, freemartins, mosaics and chimeras in animals in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 421–464.

Benirschke, K. and Driscoll, S.G. (1967) *The*

Pathology of the Human Placenta (eds K. Bernirschke and S.G. Driscoll), Springer-Verlag, New York, pp. 216.

Benirschke, K. and Robb, J.A. (1987) Infectious causes of fetal death. *Clinical Obstetrics and Gynecology*, **30**, 284–294.

Bennegard, B., Hahlin, M. and Dennefors, B. (1987) Antigonadotropic effect of oxytocin on the isolated human corpus luteum. *Fertility and Sterility*, **47**, 431–436.

Benno, R.H. and Williams, T.H. (1978) Evidence for intracellular localization of alpha-fetoprotein in the developing rat brain. *Brain Research*, **142**, 182–186.

Berg, B.N. (1967) Maintenance of pregnancy in protein-deprived rats by transitory protein supplements during early gestation. *Journal of Nutrition*, **92**, 66–70.

Bergin, W.C., Gier, H.T., Frey, R.A. and Marion, G.B. (1967) Developmental horizons and measurements useful for age determination of equine embryos and fetuses in *Proceedings of the Thirteenth Annual American Association of Equine Practitioners Meetings*, (ed. F.J. Milne), Am. Assoc. Equine Pract. Lexington, KY. pp. 179–196.

Berry, R.J. (1981) Town mouse, country mouse: Adaptation and adaptability in *Mus domesticus* (*M. musculus domesticus*). *Mammal Review*, **11**, 91–136.

Berstein, R.L. (1981) Abruptio placentae in *Principles and Practice of Obstetrics and Perinatology* (eds L. Iffy and H.A. Kaminetzky), John Wiley, New York, pp. 1085–1104.

Betterridge, K.J. and Mitchell, D. (1974) Embryo transfer in cattle: Experience of twenty-four completed cases. *Theriogenology*, **1**, 69-82.

Biggers, J.D. (1983) Risks of *in vitro* fertilisation and embryo transfer in humans in *In vitro Fertilisation and Embryo Transfer*, (ed P.G. Crossignani), Academic Press, London, pp. 393–410.

Binkerd, P.E., Hendrickx, A.G., Rice, J.M. and Palmer, A.E. (1984) Embryonic development in *Erythrocebus patas*. *American Journal of Primatology*, **6**, 15–29.

Binkerd, P.E., Tarantal, A.F. and Hendrickx, A.G. (1988) Embryonic/fetal loss and spontaneous malformations in nonhuman primates, in *Developmental Biology and Toxicology of Non-Human Primates* (eds D. Nuebert, H.J. Merker and A.G. Hendrickx), Springer-Verlag, Berlin.

Birnholz, J.C. (1986) Biological foundations for fetal growth studies in *Quantitative Obstetrical Ultrasonography* (eds R.L. Deter, R.B. Harrist, J.C. Birnholz and F.P. Hadlock), John Wiley & Sons, New York, pp. 49–64.

Blaha, G.C. (1964) Effect of age of the donor and recipient on the development of transferred golden hamster ova. *Anatomical Record*, **150**, pp. 413–416.

Blanchard, M.G. and Josso, N. (1974) Source of the anti-Müllerian hormone synthesized by the fetal testis: Müllerian-inhibiting activity of fetal bovine Sertoi cells in tissue culture. *Pediatric Research*, **8**, 968–971.

Blandau, R.J. (1961) Biology of eggs and implantation in, *Sex and Internal Secretions*, (ed. W.C. Young), Wilkins, Baltimore.

Blandau, R.J. (1973) Gamete transport in the female mammal, in *Handbook of Physiology*, Vol. II, section 7 (ed R.O. Greep), Williams & Wilkins, Baltimore, pp. 153–163.

Boatman, D.E. (1987) *In vitro* growth of nonhuman primate pre- and peri-implantation embryos, in *Mammalian Preimplantation Embryo: Regulation of Growth and Differentiation in vitro*, (ed B.D. Bavister), Plenum Press, New York, pp. 273–308.

Bodkhe, R.R., Harper, M.J.K. (1973) Mechanism of egg transport: changes in amount of adrenergic transmitter in the genital tract of normal and hormone-treated rabbits, in *Regulation of Mammalian Reproduction* (eds S.J. Segal, R. Crozier, P.A. Corfman and P.G. Condliffe), Thomas, Springfield, Illinois, pp. 364–375

Bodyazhina, V.I., and Roganova, K.G. (1966) The condition of the foetuses in pregnant animals under the treatment of oestrogens and progesterone, *Akusherstvo i Ginekologiya*, **6**, 17–23.

Bomsel-Helmreich, O. (1965) Heteroploidy and embryonic death, in *Preimplantation Stages of Pregnancy* (eds G.E.W. Wolstenholme and M. O'Connor), Little, Brown, Boston, pp. 246–269.

Bonduelle, M. L., Dodd, L., Liebaars, I., Van Steirteghem, A., Williamson, R. and Akhurst, R. (1988) Chorionic gonadotrophin-β mRNA, a trophoblast market, is expressed in human 8 cell embryos derived from tripronucleate zygotes. *Human Reproduction*, **3**, 909–914.

Bongiovanni, A. M. (1962) The adrenogenital syndrome with deficiency of 3R-hydroxysteroid dehydrogenase. *Journal of Clinical Investigation*, **41**, 2086–2092.

Bongiovanni, A. (1978) Congenital adrenal hyperplasia and related conditions, in *The Metabolic*

Basis of Inherited Disease, (eds J.B. Stanbury, J.B. Wyngarden and D.S. Fredericksen), McGraw-Hill, New York, pp. 868.

Boot, R., Leussink, A.B. and Vlug, R.F. (1985) Influence of housing conditions on pregnancy outcome in cynomolgus monkeys (*Macaca fascicularis*). *Laboratory Animals*, **19**, 42–47.

Boué, A., Boué, J. and Gropp, A. (1985) Cytogenetics of pregnancy wastage, in *Advances in Human Genetics*, Vol. 14, (eds H. Harris and K. Hirschhorn), Plenum Press, New York, pp. 1–57.

Boué, J., Boué, A. and Lazar, P. (1975) Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. *Teratology*, **12**, 11–26.

Bousfield, G.R., Liu, W.K., Sugino, H. and Ward, D.N. (1987) Structural studies of equine glycoprotein hormones. Amino acid sequence of equine lutropin β-subunit. *Journal of Biological Chemistry*, **262**, 8610–8620.

Boving, B. (1963) Implantation mechanisms, in *Conference on Physiological Mechanisms Concerned with Conception*, (ed. C.G. Hartman), Pergamon Press, London, pp. 321–396.

Bowden, R.S.T., Hodgman, S.F.J. and Hime, J.M. (1963) Neonatal mortality in dogs. *World Veterinary Congress Proceedings*, 17th Congress, Vol. 2, 1009–1013. Ebner and Seubert, Stuttgart.

Bowie, J.D. (1988) Fetal growth, in *Ultrasonography in Obstetrics and Gynecology*, (ed. P.W. Callen), 2nd edn, W.B. Saunders, Philadelphia, pp. 65–82.

Boyd, H., Bacsich, P., Young, A. and McCraken, J.A. (1969) Fertilization and embryonic survival in dairy cattle. *British Veterinary Journal*, **125**, 87–97.

Boyd, J.D. and Hamilton, W.J. (1964) Cleavage, early development and implantation of the egg, in *Marshall's Physiology of Reproduction*, 3rd edn, Vol. 2, (ed. A.S. Parkes), Longmans, London, pp. 1–126.

Boyd, J.D. and Hamilton, W.J. (1970) *The Human Placenta*, Heffer, Cambridge.

Bramley, T.A., Menzies, G.S. and Baird, D.T. (1985) Specific binding of gonadotrophin-releasing hormone and an agonist to human corpus luteum homogenates: characterization, properties and luteal phase levels. *Journal of Endocrinology and Metabolism*, **61**, 834–841.

Bramley, T.A., Stirling, D., Swanston, I.A., Menzies, G.S., McNeilly, A.S. and Baird, D.T. (1987) Specific binding sites for gonadotrophin-releasing hormone, LH/chorionic gonadotrophin, low-density lipoprotein, prolactin and FSH in homogenates of human corpus luteum. I. Concentrations throughout the luteal phase of the menstrual cycle and early pregnancy. *Journal of Endocrinology*, **113**, 317–327.

Branch, D.W. (1987) Immunologic disease and fetal death. *Clinical Obstetrics and Gynecology*, **30**, 295–311.

Braustein, G.D., Grodin, J.M., Vaitukaitis, J. and Ross, G.T. (1973) Secretory rates of human chorionic gonadotropin by normal trophoblast. *American Journal of Obstetrics and Gynecology*, **115**, 447–451.

Breedlove, S.M. (1984) Steroid influences on the development and function of a neuromuscular system, in *Progress in Brain Research*, Vol. **61**, (eds G.J. deVries, J.P.C. deBruin, H.B.M. Uylings and M.A. Corner), Elsevier, Amsterdam, pp. 147–169.

Brent, R.C. (1977) Radiations and other physical agents, in *Handbook of Teratology: 1. General Principles and Etiology* (eds J.G. Wilson and F.C. Fraser), Plenum Press, New York pp. 153–223.

Brinster, R.L. (1974) Embryo development. *Journal of Animal Science*, **38**, 1003–1012.

Brown H.L. (1991) Antiphospholipid antibodies and recurrent pregnancy loss. *Clinical Obstetrics and Gynecology*, **34**, 17–26.

Brown, N.A. and Fabro, S. (1983) The value of animal teratogenicity testing for predicting human risk. *Clinical Obstetrics and Gynecology*, **26**, 467–477.

Brown, T.R. and Migeon, C.J. (1986) Androgen receptors in normal and abnormal male sexual differentiation. *Advances in Experimental Medicine and Biology*, **196**, 227–255.

Browne, J.C. and Veall, N. (1953) The maternal–placental blood flow in normotensive and hypertensive women. *Journal of Obstetrics and Gynaecology of the British Empire*, **60**, 141–147.

Bruce, N.W. and Bartholomeusz, R.K. (1976) The effects of medroxyprogesterone acetate on the maintenance of pregnancy in ovariectomized rats and rabbits. *Teratology*, **13**, 309–316.

Bruere, A.N., MacDonald, M.F. and Marshall, R.B. (1969) Cytogenetical analysis of an ovine male pseudohermaphrodite and the possible role of the Y chromosome in crypotorchidism of sheep. *Cytogenetics*, **8**, 148–157.

Bulmer, D. (1957) The development of the human vagina. *Journal of Anatomy*, **91**, 490–509.

Burchell, R.C., Creed, F., Rasoulpour, M. and

Whitcomb, M. (1978) Vascular anatomy of the human uterus and pregnancy wastage. *British Journal of Obstetrics and Gynaecology*, **85**, 698–706.

Burgess, A.W. (1989) Epidermal growth factor and transforming growth factor alpha. *British Medical Bulletin*, 45, 401–424.

Burgoyne, P.S. (1988) Role of mammalian Y chromosome in sex determinations. *Philosophical Transactions of the Royal Society of London*, Series **322 (1208)**, 63–72.

Burgoyne, P.S., Levy, R.E. and McLaren, A. (1986) Spermatogenic failure in male mice lacking H-Y antigen. *Nature*, **320**, 170.

Butler, H. (1983) The embryology of the lesser galago (*Galago senegalensis*) in *Contributions to Primatology*, (ed. F.S. Szalay), Basle, Karger, pp. 1–56.

Cameron, J.L. and Stouffer, R.L. (1982) Gonadotropin receptors of the primate corpus luteum. I. Characterization of the 125-I-labeled human luteinizing hormone and human chorionic gonadotropin binding to luteal membranes from the rhesus monkey. *Endocrinology*, **110**, 2059–2067.

Campbell, R.M., Innes, I.R. and Kosterlitz, H.W. (1953) Same dietary and hormonal effects on maternal, foetal and placental weights in the rat. *Journal of Endocrinology*, **9**, 68–75.

Canfield, R.E., Morgan, F.J., Kammerman, S., Bell, J.J. and Agosto, G.M. (1971) Studies of human gonadotrophin. *Recent Progress in Hormone Research*, **27**, 121–156.

Carr, D.H. (1967) Chromosome anomalies as a cause of spontaneous abortion. *American Journal of Obstetrics and Gynecology*, **97**, 283–293.

Carr, D.H., Haggar, R.A. and Hart, A.G. (1968) Germ cells in the ovaries of XO female infants. *Annual Journal Clinical Pathology*, **19**, 521–526.

Casper, R.F., Erickson, G.F., Rebar, R.W. and Yen, S.S.C. (1982) The effect of luteinizing hormone-releasing factor and its agonist on cultured human granulosa cells. *Fertility and Sterility*, **37**, 406–409.

Cassady, G. (1981) The small-for-date infant, in *Neonatology: Pathophysiology and Management of the Newborn*, (ed. G. Avery), J.B. Lippincott, Philadelphia, pp. 262–286.

Cattanach, B.M. (1985) Information on the Y chromosome and sex determination from mutants and other variants of sexual development. *Archives D Anatomie Microscopique et de Morphologie Experimentale*, **74**, 5–32.

Centerwall, W.R. and Benirschke, K. (1973) Male tortoiseshell and Calico (T-C) cats. *Journal of Heredity*, **64**, 272–278.

Chambers, P.L. and Hearn, J.P. (1979) Peripheral plasma levels of progesterone, oestradiol 17β, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey *Callithrix jacchus*. *Journal of Reproduction and Fertility*, **56**, 23–32.

Chambers, P.L. and Hearn, J.P. (1985) Embryonic feotal and placental development in the common marmoset monkey (*Callithrix jacchus*). *Journal of Zoology*, (*London*), A, **207**, 545–561.

Chameides, L., Truex, R.C., Vetter, V., Rashkind, W.J., Galioto, F.M. and Noonan, J.A. (1977) Association of maternal systemic lupus erythematosus with congenital complete heart block. *New England Journal of Medicine*, **297**, 1204–1207.

de la Chapelle, A. (1981) The etiology of maleness in XX men. *Human Genetics*, **58**, 105–116.

de la Chapelle, A, Tippett, R.A., Wetterstrand, G. and Page, D. (1984) Genetic evidence of X-Y interchange in a human XX male. *Nature*, **307**, 170–171.

Chapman, M., Grudzinskas, G. and Chard, T. (1988) *Implantation*, Springer-Verlag, Berlin.

Charpigny, G., Reinaud, P., Huet, J.C., Guillomot, M., Charlier, M., Pernallet, J.C. and Martal, J. (1988) High homology between trophoblastic protein (trophoblastin) isolated from ovine embryo and α-interferons. *Federal European Biochemical Society*, **228**, 12–16.

Chatterjee, M. and Munro, H.N. (1977) Structure and biosynthesis of human placental peptide hormones. *Vitamins and Hormones*, **35**, 149–208.

Cheek, D.B. and Hill, D.E. (1975) Changes in somatic growth after ablation of maternal or fetal pancreatic beta cells, in *Fetal and Postnatal Cellular Growth*, (ed. D.B. Check), Wiley, New York, Chapter 18, pp. 311–321.

Chen, T.T., Bazer, F.W., Gebhardt, B. and Roberts, R.M. (1975) Uterine secretion in mammals; synthesis and placental transport of a purple acid phosphatase in pigs. *Biology of Reproduction*, **13**, 304–313.

Chernoff, G.F. (1977) The fetal alcohol syndrome in mice: an animal model. *Teratology*, **15**, 223–230.

Chernoff, N. and Rogers, E.H. (1976) Fetal toxicity of kepone in rats and mice. *Toxicology and Applied Pharmacology*, **38**, 189–194.

Chernoff, W. (1973) Teratogenic effects of cadmium in rats. *Teratology*, **8**, 29–32.

Cho, F., Hanari, K., Suzuki, M. and Honjo, S.

(1985) Relationship between fetal position and stillbirth in the cynomolgus monkeys (*Macaca fascicularis*). Retrospective analysis. *Journal of Medical Primatology*, **14**, 169–174.

Clayton, R.N., Harwood, J.P. and Catt, K.J. (1979) Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. *Nature* (London), **282**, 90–92.

Clemens, L.G. (1974) Neurohormonal control of male sexual behavior, in *Reproductive Behavior*, (eds W. Montagna and W.A. Sadler), Plenum Press, New York, pp. 23–53.

Clemens, L.G. and Coniglio, L. (1971) Influence of prenatal litter composition on mounting behavior of female rats. *American Zoologist*, **11**, 617–618.

Cocchiara, R., Tripani, G., Azzolina, A., Albeggiani, G., Cirimmina, R., Cephalu, E., Cittadini, E. and Geraci, D. (1987) Isolation of a histamine releasing factor from human embryo culture medium after *in vitro* fertilisation. *Human Reproduction*, **2**, 341–344.

Connors, T.J., Thorpe, L.W. and Soderwall, A.L. (1972) An analysis of preimplantation embryonic death in senescent golden hamsters. *Biology of Reproduction*, **6**, 131–135.

Corbett, T.H., Beaudoin, A.R., Cornell, R.G., Anver, M.R., Schumacher, R., Endres, J. and Szwobowska, M. (1975) Toxicity of polybrominated biphenyls (Firemaster BP-6) in rodents. *Environmental Research*, **10**, 390–396.

Corker, C.S., Michie, E., Hobson, B. and Parboosingh, J. (1976) Hormonal patterns in conceptual cycles and early pregnancy. *British Journal of Obstetrics and Gynaecology*, **83**, 489–494.

Cotton, D.B., Read, J.A., Paul, R.H. and Quilligan, E.J. (1980) The conservative aggressive management of placenta previa. *American Journal of Obstetrics and Gynecology*, **137**, 687–695.

Courtney, K.D. and Valerio, D.A. (1968) Teratology in the *Macaca mulatta*. *Teratology*, **1**, 163–172.

Coyle, I.R. and Anker, R. and Cragg, B. (1976) Behavioral, biochemical and histological effects of prenatal administration of progesterone in the rat. *Pharmacology, Biochemistry and Behavior*, **5**, 587–590.

Croxatto, H.B. and Ortiz, M.S. (1975) Egg transport in the fallopian tube. *Gynecological Investigation*, **6**, 215–225.

Csapo, A.I., Pulkkinen, M.O., Ruttner, B., Sauvage, J.P. and Wiest, W.G. (1972) The significance of the human corpus luteum in pregnancy maintenance. *American Journal of Obstetrics and Gynecology*, **112**, 1061–1067.

Cunha, G.R. (1975) Age-dependent loss of sensitivity of female urogenital sinus to androgenic conditions as a function of the epithelial–stromal interaction in mice. *Endocrinology*, **97**, 665–673.

Cunha, G.R. and Lung, B. (1979) The importance of stroma in morphogenesis and functional activity of urogenital epithelium. *In Vitro*, **15**, 50–71.

Cupceancu, B. and Neumann, F. (1969) Sensibilitaetsunterschiede der verschiedenen Strukturen des Genitaltyraktes von weiblichen Rattenfoeten unter dem Einfluss von Medroxyprogesteronacetat oder Norethisteronacetat. *Endokrinologie*, **54(1/2)**, 66–80.

Curtis, E.M. and Grant, R.P. (1964) Masculinization of female pups by progestogens. *Journal of the American Veterinary Medical Association*, **144**, 395–398.

Dain, A.R. and Walker, R.G. (1979) Two intersex dogs with mosaicism. *Journal of Reproduction and Fertility*, **56**, 239–242.

Dasgupta, P.R., Srivastava, K. and Kar, A.B. (1973) Effects of d-Norgestrel on early pregnancy in rats. *Indian Journal of Experimental Biology*, **11**, 321–322.

Davies, J. and Hesseldahl, J. (197l) Comparative embryology of mammalian blastocysts, in *The Biology of the Blastocysts* (ed. R.J. Brinster), University of- Chicago Press, Chicago, Illinois, pp. 27.

Davies, J., Russell, M. and Davenport, G.R. (1985) Effects of maternal administration of diethylstilbestrol and estradiol on the newborn guinea pig. *Acta Anatomica*, **122(1)**, 39–61

Davis, R.M. (1981) Localisation of male determining factors in man: A thorough review of structural anomalies of the Y chromosome. *Journal of Medical Genetics*, **18**, 161–195.

Dawes, G.S. (1973) *Foetal and Neonatal Physiology*, New York Medical Publisher, Chicago.

Dawes, G.S. (1976) The physiological determinants of fetal growth. *Journal of Reproduction and Fertility*, **47**, 183–187.

Dawood, M.Y. and Khan-Dawood, F.S. (1986) Human ovarian oxytocin. Its source and relationship to steroid hormones. *American Journal of Obstetrics and Gynecology*, **154**, 756–763.

Day, B.N. (1962) The reproduction of swine, in *Reproduction in Farm Animals*, (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 255–265.

Delahunt, C.S. and Rieser, N. (1967) Rubella-

induced embryopathies in monkeys. *American Journal of Obstetrics and Gynecology*, **99**, 580–588.

Dennefors, B.L., Sjorgen, A. and Hamberger, L. (1982) Progesterone and adenosine 3′,5′-monophosphate formation by isolated human corpus luteum at different ages: influence of human chorionic gonadotrophin and prostaglandins. *Journal of Clinical Endocrinology and Metabolism*, **55**, 102–107.

D'Ercole, A.J. and Underwood, L.E. (1986) Regulation of fetal growth by hormones and growth factors in *Human Growth:A Comprehensive Treatise*, Vol. l, (eds F. Falkner and J.M. Tanner), Plenum Press, New York, London, pp. 327–338.

Dixson, A.F. (1983) The owl monkey (*Aotus trivirgatus*). In: Hearn, J.P. (ed), *Reproduction in New World Primates*. MTP Press, Lancaster, pp 69–115.

Dockras, A., Sargent, I.L., Ross, C., Gardner, R.L. and Barlow, D.H. (1991) The human blastocyst: morphology and human chorionic gonadotrophin secretion *in vitro*. *Human Reproduction*, **6**, 1143–1151.

Dohler, K.D., Hancke, J.G., Srivastava, S.S., Hofmann, C, Shryne, J.E. and Gorski, R.A. (1984a) Participation of estrogens in female sexual differentiation of the brain: Neuroanatomical, neuroendocrine and behavioral evidence, in *International Summer School of Brain Research* (eds F.J. deVries, J.P.C. deBruin, H.B.M. Uylings and M.A. Corner), Brain Research, Vol. **61**, Elsevier, Amsterdam, pp. 99–117.

Dohler, K.D., Coquelin, F.D., Hines, M., Shryne, J.E. and Gorski, R.A. (1984b) Pre- and post-natal influence of testosterone propionate and diethylstilbestrol on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. *Brain Research*, **302**, 291–295.

Donahoe, P.K., Ito, Y., Morikawa, Y. and Hardy Hendren W. (1977) Müllerian inhibiting substance in human testes after birth. *Journal of Pediatric Surgery*, **12**, 323–330.

Drost, M. and Holm, L.W. (1968) Prolonged gestation in ewes after foetal adrenalectomy. *Journal of Endocrinology*, **40**, 293–296.

Drumm, M., Jung, G. and Erickson, E.P. (1985) Isolation of human X-chromosomal clones homologous to sex-related BKM sequences. *American Journal of Human Genetics*, **37(4)**, A125.

Dukelow, W.R. (1983) The squirrel monkey, in *Reproduction in New World Primates* (ed. J.P. Hearn), Lancaster MTP Press, Lancaster, pp. 149–180.

Dunn, H.O., Kenney, R.M. and Lein, D.H. (1968) XX/XY chimerism in a bovine true hermaphrodite: An insight into the understanding of freemartinism. *Cytogenetics*, **7**, 390–402.

Dunn, H.O., McEntee, K. and Hansel, W. (1970) Diploid/triploid chimerism in a bovine true hermaphrodite. *Cytogenetics*, **9**, 245–259.

Dutt, R.H. (1960) Temperature and light as factors in reproduction among farm animals. *Journal of Dairy Science*, **43** (Suppl), 123–144.

Dutt, R.H. and Simpson, E.C. (1957) Environmental temperature and fertility of southdown rams early in the breedng season. *Journal of Animal Science*, **6**, 136–143.

Dyban, A.P. and Baranov, V.S. (1987) *Cytogenetics of Mammalian Embryonic Development*, Clarendon Press, Oxford, pp. 227–241.

Dyche, W.J. (1979) A comparative study of the differentiation and involution of the mullerian duct and wolffian duct in the male and female fetal mouse. *Journal of Morphology*, **162**, 175–210.

Dziuk, P.J. and Henshaw, G. (1958) Fertility of boar semen artificially inseminated following *in vitro* storage. *Journal of Animal Science*, **17**, 554–558.

Earl, F.L. (1978) Dogs (developmental abnormalities), in *Pathology of Laboratory Animals*, (eds K. Benirschke, F.M. Garner and T.C. Jones), Vol. 2, Springer-Verlag, New York, pp. 1874–1886.

Eaton, O.N. (1943) An anatomical study of hermaphroditism in goats. *American Journal of Veterinary Research*, **4**, 333–343.

Eckstein, P., McKeown, T. and Record, R.G. (1955) Variation in placental weight according to litter size in the guinea pig. *Journal of Endocrinology*, **12**, 108–114.

Eddy, C.A., Garcia, R.G., Kraemer, D.C. and Paverstein, C.J. (1975) Ovum transport in non-human primates, in *Symposium on Ovum Transport and Fertility Regulation. IV. Control of Ovum Transport in the Oviduct*, Scriptor, Copenhagen, pp. 390–403.

Eden, A.N. (1966) Perinatal morbidity caused by *Vibrio fetus*. *Journal of Pediatrics*, **68**, 297–304.

Edey, T.N. (1967) Early embryonic death and subsequent cycle length in the ewe. *Journal of Reproduction and Fertility*, **13**, 437–443.

Edey, T.N. (1976) Embryo mortality, in *Sheep Breeding*, (eds G.J. Tomes, E.E. Robertson and R.J. Lightfoot), Proceedings of the International

Congress, Muresk, Western Australia Institute of Technology, Perth, pp. 400–410.

Edgren, R.A. and Clancy, D.P. (1968) The effects of Norgestrel, Ethinyl Estradiol, and their combination (Ovral) on the young of female rats treated during pregnancy. *International Journal of Fertility*, **13**, 209–214.

Edwards, R.G. (1980) *Conception in the Human Female*, Academic Press, London.

Edwards, R.G. (1985) Current status of human conceptions *in vitro*. *Proceedings of the Royal Society of London*, **B 223**, 417–448.

Edwards, R.G., Purdy, J.M. and Steptoe, P.C. (eds) (1985) *Implantation of the Human Embryo*, Academic Press, London.

Ehrhardt, A. and Money, J. (1967) Progestin-induced hermaphroditism: IQ and psychosexual identity in a study of ten girls. *Journal of Sex Research*, **3**, 83–100.

Eicher, E.M. (1988) Autosomal genes involved in primary sex determination. *Philosophical Transactions of the Royal Society of London*, Series **322 (1208)**, 109–118.

Eichwald, E.J. and Silmser, C.R. (1955) *Transplantation Bulletin*, **2**, 148–149.

Einer-Jensen, N., Bendz, A. and Leidenberger, F. (1985) Counter-current exchange of 13C-progesterone in the human ovarian adnex. *Acta Endocrinologica*, **108**, 102.

Eley, R.M., Thatcher, W.W., Bazer, F. W., Wilcox, C.J., Becker, R.B., Head, H.H. and Adkinson, R.W. (1978) Development of the conceptors in the bovine. *Journal of Dairy Science*, **61(4)**, 467–473.

Eley, R.M., Summers, P.M. and Hearn, J.P. (1987) Failure of prostaglandin $F_{2\alpha}$ analogue, cloprostenol to induce functional luteolysis in the olive baboon (*Papio cynocephalus anubis*). *Journal of Medical Primatology*, **16**, 1–12.

Ellinwood, W.E., Normal, R.L. and Spies, H.G. (1984) Changing frequency of pulsatile luteinizing hormone and progesterone secretion during the luteal phase of the menstrual cycle of rhesus monkeys. *Biology of Reproduction*, **31**, 714–722.

Ellis, S.J., Heap, R.B., Butchart, A.R., Rider, U., Richardson, N.E., Wang, M.Y. and Taussig, M.J. (1988) Efficiency and specificity of antiprogesterone monoclonal antibodies in preventing the establishment of pregnancy in the mouse. *Journal of Endocrinology*, **118**, 69–80.

El-Sheikh, A.S., Hulet, C.V., Pope, A.L. and Casida, L.E. (1955) The effect of level of feeding on the reproductive capacity of the ewe. *Journal of Animal Science*, **14**, 919–929.

Enders, A.C. (1972) Mechanisms of implantation of the blastocyst, in *Biology of Reproduction, Basic and Clinical Studies* (eds J. Velardo and B. Kasprow), Pan American Congress of Anatomy, New Orleans, Louisiana, pp. 313–334.

Enders, A.C. and Hendrickx, A.G. (1980) Implantation in non human primates in *Non Human Primate Models for Study of Human Reproduction* (ed T.C. Anand Kumar), Karger, Basle, pp. 98–108.

Enders, A.C., Hendrickx, A.G. and Binkerd, P.E. (1982) Abnormal development of blastocysts and blastomeres in the rhesus monkey. *Biology of Reproduction*, Vol. 26, 353–366.

Enders, A.C., Hendrickx, A.G. and Schlafke, S. (1983) Implantation in the rhesus monkey: Initial penetration of endometrium. *American Journal of Anatomy*, **167**, 275–298.

Environmental Protection Agency (EPA) (1986): Final guidelines for the health assessement of suspect developmental toxicants. *Federal Register*, **51**, 34 028–34 040.

Erb, R.E. and Holtz, E.W. (1958) Factors associated with estimated fertilization and service efficiency of cows. *Journal of Dairy Science*, **41**, 1541–1552.

Evans, E.P., Ford, C.F. and Lyon, M.F. (1977) Direct evidence of the capacity of the XY germ cell in the mouse to become an oocyte. *Nature*, **267**, 430–431.

Evans, H.E. and Sack, W.O. (1973) Prenatal development of domestic and laboratory mammals. *Anatomia, Histologia, Embryologia*, **2**, 11–45.

Fairclough, R.J., Smith, J.F. and McCovan, L.T. (1981) Prolongation of the oestrous cycle in cows and ewes after passive immunisation with PGF antibodies. *Journal of Reproduction and Fertility*, **62**, 213–219.

Farrer-Brown, G., Bellby, J.W. and Tarbet, M.H. (1971) Venous changes in the endometrium of myomatous uteri. *Obstetrics and Gynecology*, **38**, 743–751.

Fechheimer, N.S. and Beatty, R.A. (1974) Chromosomal abnormalities and sex ratio in rabbit blastocysts. *Journal of Reproductive Fertility*, **37**, 331–341.

Fehilly, C.B., Willadsen, S.M. and Tucker, E.M. (1984) Interspecific chimaerisms between sheep and goat. *Nature* **307**, 634–636.

Felding, C. (1968) Pregnancy following renal

diseases. *Clinical Obstetrics and Gynecology*, **11**, 579–593.

Feldman, K.W. and Smith, D.W. (1975) Fetal phallic growth and penile standards for newborn male infants. *Journal of Pediatrics*, **86**, 395–398.

Ferm, V.H. (1972) The teratogenic effects of metals on mammalian embryos. *Advances in Teratology*, **5**, 51–75.

Ferm, V.H. and Carpenter, S.J. (1967) Teratogenic effect of cadmium and its inhibition by zinc. *Nature*, **216**, 1123.

Ferreira, G.F., Rega, R.M. and Mandarim-de-Lacerda, C.A. (1990) Allometry of hepatic weight growth in human staged fetuses. *Archivio Italiano di Anatomia E di Embriologia*, **95**, 223–228.

Ferreira, G.F., Rega, R.M. and Mandarim-de-Lacerda, C.A. (1992) Spleen's relative growth in human fetuses. *Bulletin de L'Association de Anatomists*, **76**, 23–25.

Filicori, M., Butler, J.P. and Crowley, W.F. (1984) Neuroendocrine regulation of the corpus luteum in the human. *Journal of Clinical Investigation*, **3**, 1638–1647.

Findlay, J.K., Cerini, M., Sheen, M., Staples, L.D. and Cumming, I.A. (1979) The nature and role of pregnancy associated antigens and the endocrinology of early pregnancy in the ewe. *Ciba Symposium*, **64**, 239–260.

Finn, C.A. (1962) Embryonic death in aged mice. *Nature*, **194**, 499–500.

Finn, C.A. (1985) Uterine responses to the corpus luteum, in *The Luteal Phase* (ed. S.L. Jeffcoate), John Wiley & Sons, Chichester, pp. 43–59.

Fisch, B., Margara, R.A., Winston, R.M.L. and Hillier, S.G. (1989) Cellular basis of luteal steroidogenesis in the human ovary. *Journal of Endocrinology*, **122**, 303–311.

Fishel, S.B., Edwards, R.G. and Evans, C. (1984) Human chorionic gonadotrophin secreted by preimplantation embryos cultured *in vitro*. *Science*, New York, **223**, 816–818.

Fletcher, P.W. and Niswender, G.D. (1982) Effect of $PGF_{2\alpha}$ on progesterone secretion and adenylate cyclase activity in ovine luteal tissue. *Prostaglandins*, **6**, 803–818.

Flickinger, C.J. (1969) Fine structure of the Wolffian duct and cyto-differentiation of the epididymis in fetal rats. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **96**, 344–360.

Flickinger, C.J. (1970) The fine structure and development of the seminal vesicles and prostate in the fetal rat. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **109**, 1–14.

Flint, A.P.F. and Hillier, K. (1975) Prostaglandins and reproductive processes in female sheep and goats, *Prostaglandins and Reproduction* (ed. S.M.M. Karim), MTP Press Ltd, Lancaster, pp. 271–308.

Flint, A.P.F. and Sheldrick, E.L. (1986) Ovarian oxytocin and the maternal recognition of pregnancy. *Journal of Reproduction and Fertility*, **76**, 831–839.

Flint, A.P.F., Buxton, R.D., Gadsby, J.E., Saunders, P.T.R. and Heap, R.B. (1979) Blastocyst oestrogen synthesis and the maternal recognition of pregnancy, in *Maternal Recognition of Pregnancy* (ed. J. Whelan), Ciba Foundation Symposium No. 64, Excerpta Medica, Amsterdam, pp. 209–228.

Flint, A.P.F., Lamming, G.E. and Stewart, H.J. (1988) A role for interferons in the maternal recognition of pregnancy. *Molecular and Cellular Endocrinology*, **58**, 109–111.

Flint, A.P.F., Stewart, H.J., Lamming, G.E. and Payne, J.H. (1992) Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants, in *Journal of Reproduction and Fertility*, Suppl. 45, pp. 53–56

Food and Drug Administration (FDA) (1966): *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use*, Drug Review Branch, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration Washington, DC.

Foote, W.C., Pope, A.L., Chapman, A.B. and Casida, L.E. (1959) Reproduction in the yearling ewe as affected by breed and sequence of feeding levels. I. Effects on ovulation rate and embryo survival. *Journal of Animal Science*, **18**, 453–462.

Foote, W.D., Foote, W.C., and Foote, L.H. (1968) Influence of certain natural and synthetic steroids on genital development in guinea pigs. *Fertility and Sterility*, **19**, 606–615.

Ford, J.J. and D'Occhio, M.J. (1989) Differentiation of sexual behavior in cattle, sheep and swine. *Journal of Animal Science*, **67**, 1816–1823.

Ford, N. (1988) *When Does Life Begin?* Cambridge University Press, Cambridge.

Ford, C.E., Evans, E.P., Burtenshaw, M.D., Clegg, H.M., Tuffrey, M. and Barnes, R.D. (1975) A functional 'sex-reversed' oocyte in the mouse. *Proceedings of the Royal Society of London*, B 190, 187–197.

Forsberg, J.C. (1965) Origin of vaginal epithelium. *Obstetrics and Gynecology*, **25**, 787–791.

Fraedrich, J. (1979) Licht- und elektronenmikroskopische untersuchungen uber den zusammenhang der mesonephros- und fruhen gonaden-entwicklung der weissen Maus. *Dissertation*, Med Fak Bonn, pp. 1–58.

Fraga, A., Mintz, G., Orozco, J.H. and Orozco, J. (1973) Systemic lupus erythematosus: Fertility, fetal wastage and survival rate with treatment. A comparative study. *Arthritis and Rheumatism*, **16**, 541.

Fralick, R.L. and Murray, R.C. (1948) Pseudohermaphroditism in an adult dog. *Anatomical Record*, **100**, 741–742.

Frank, L. and Roberts, R.J. (1979) Effects of low-dose prenatal corticosteroid administration on the premature rat. *Biology of the Neonate*, **36**, 1–9.

Fraser, H.M., Baird, D.T., McRae, G.I., Nestor, J.J. and Vickery, B.H. (1985) Suppression of luteal progesterone secretion in the stumptailed macaque by an antagonist analogue of luteinising hormone-releasing hormone. *Journal of Endocrinology*, **104**, R1–R4.

Fraser, H.M., Abbott, M., Laird, N.C., McNeilly, A.S., Nestor, J.J. and Vickery, B.M. (1986) Effects of an LHRH antagonist on the secretion of LH, FSH, prolactin and ovarian steroids at different stages of the luteal phase in the stmptailed macaque (*Macaca arctoides*). *Journal of Endocrinology*, **111**, 83–89.

Fredga, K. (1972) Comparative chromosome studies in mongooses (*Carnivora, Viverridae*). *Mereditos*, **71**, 1–74.

Fredga, K., Gropp, A., Winking, H. and Frank, F. (1976) Fertile XX- and XY- type females in the wood lemming, *Myopus schisticolor*. *Nature*, **261**, 225–227.

French, F.E. and Bierman, J.M. (1962) Probabilities of fetal mortality. *Public Health Report*, **77**, 835–847.

Freire, J.R.C. (1955) Efeito do benzoato de estradiol sobre a gestagao em ratos. 1. Influencia da dose do 40 ao 90 dia de gestago. *Annales de la Academia de Ciencias* (Brazil), **27**, 77–78.

Fylling, P., Sjaastad, O.V. and Velle, W. (1973) Midterm abortion induced in sheep by synthetic steroids. *Journal of Reproduction and Fertility*, **32**, 305–306.

Gabriel-Robez, O., Rohmer, A., Clavert, J. and Schneegans, E. (1972) Actions tératogène et lethale du diethylstillboestrol sur l'émbryon de souris. *Archives Françaises de Pédiatrie*, **29**, 149–154.

Gabrilove, J.L. (1989) Introduction and overview of hematopoietic growth factors. *Seminars in Hematology*, **26(2 Supp 2)**, 1–4.

Gadsby, J.E., Heap, R.B. and Burton, R.D. (1980) Oestrogen production by blastocyst and early embryonic tissue of various species. *Journal of Reproduction and Fertility*, **60**, 409–417.

Gale, T.F. (1978) Embryotoxic effects of chromium trioxide in hamsters. *Environmental Research*, **16**, 101–109.

Gale, T.F. and Ferm, V.H. (1971) Embryopathic effects of mercuric salts. *Life Sciences*, **10**, 1341–1347.

Gandolfi, F., Moor, R.M. (1987) Stimulation of early embryonic development in the sheep by co-culture with oviduct epithelial cells. *Journal of Reproduction and Fertility*, **81**, 23–28.

Garrow, F. and Pentschew, A. (1964) Neonatal hydrocephalus in the offspring of rats fed during pregnancy non-toxic amounts of tellurium. *Archiv fuer Psychiatric und Nervenkrenkrankheiten*, **206**, 272–280.

Gasser, D.L. and Silvers, W.K. (1972) Genetics and immunology of sex-linked antigens. *Advances in Immunology*, **15**, 215–247.

Gasser, R.F. (1985) The prenatal development of the cervix, vagina, and vulva. *The Colposcopist*, **XVII(3)**, 1–10.

Gebhardt, D.O.E. (1970) The embryolethal and teratogenic effects of cyclophosphamide on mouse embryos. *Teratology*, **3**, 273–278.

Geisert, R.D., Biggers, B., Wettemann, R.P. and Zavy, M.T. (1984) Length of pseudopregnancy in the gilt is influenced by days of oestradiol benzoate treatment. *Proceedings of 10th International Congress of Animal Reproduction & A.I.* Urbana I. Abstract 507.

Gellen, J. and Gyori, I. (1969) The weight increase of the placenta and embryo in early human pregnancy. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **76**, 990–992.

George, F.W. and Wilson, J.D. (1978) Conversion of androgen to estrogen by the human fetal ovary. *Journal of Clinical Endocrinology and Metabolism*, **47**, 550–555.

German, J., Simpson J.L., Chaganti, A.S.K., Summitt, R.L., Bruce Reid, L. and Merkatz, I.R. (1978) Genetically determined sex-reversal in 46, XY humans. *Science*, **202**, 53–56.

Gerneke, W.H., De Boom, H.P.A. and Heinichen,

I.G. (1968) Two canine intersexes. *Journal South Africna Veterinary Medicine Association*, **39**, 56–59.

Gewolb, I.H. and Warshaw, J.B. (1983) Influences on fetal growth, in *The Biological Basis of Reproductive and Developmental Medicine* (ed. J B Warshaw), Elsevier Biomedical, New York, pp. 365–389.

Gidley, J.T., Christensen, H.D., Hall, I.H., Palmer, K.H. and Wall, M.E. (1970) Teratogenic and other effects produced in mice norethynodrel and its 3-hydroxymetabolites. *Teratology*, **3**, 339–34.

Gileva, E.A. and Chebotac, N.A. (1979) Fertile XO males and females in the varying lemming, *Dicrostonyx torquatus* Pall. *Heredity*, **42**, 67–77.

Gill, T.J. (1990) Immunogenetics of recurrent abortions in experimental animals and in humans in *The Immunology of the Fetus*, (ed. G. Chaouoat), CRC Press, Boca Raton, Florida, pp. 293–306.

Gill, W.B. (1988) Effects on human males of *in utero* exposure to exogenous sex hormones in *Toxicity of Hormones in Perinatal Life* (eds T. Mori and H. Nagasawa), CRC Press, Boca Raton, Florida, pp. 161–177.

Gillam, J.S. (1955) Study of the inadequate secretion phase endometrium. *Fertility and Sterility*, **6**, 18–36.

Giovanni, A.D. and Cribiu, E.P. (1978) Le anomalie cromosomiche nel cavallo, *Equus caballus*. *Zootecnica e Nutrizione Animale*, **4**, 9–16.

Givens, J.R., Wiser, M.L., Summitt, R.L., Kerber, K.J., Anderson, R.N., Pittaway, D.E. and Fish, S.A. (1974) Familial male pseudohermaphroditism without gynecomastia due to deficient testicular 17-ketosteroid reductase deficiency. *New England Journal of Medicine*, **291**, 938–944.

Glenister, T.W. (1962) The development of the utricle and of the so-called 'middle' or 'median' lobe of the human prostate. *Journal of Anatomy*, **96**, 443–455.

Gluckman, P.D. (1989) Fetal growth: An endocrine perspective. *Acta Paediatrica Scandinavica*, Suppl **349**, 21–25.

Godkin, J.D., Bazer, F.W., Moffat, J., Sessions, F. and Roberts, R.M. (1982) Purification and properties of a major low molecular weight protein released by the trophoblast of sheep blastocysts on Day 13–21. *Journal of Reproduction and Fertility*, **65**, 141–150.

Godkin, J.D., Bazer, F.W., Thatcher, W.W. and Roberts, R.M. (1984) Proteins released by cultured Day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine of cyclic ewes. *Journal of Reproduction and Fertility*, **71**, 57–64.

Goebelsmann, U., Horton, R., Mestman, J.H., Arce, J.J., Nagata, Y., Nakamura, R.M., Thorneycroft, I. H. and Mishell, D.R. (1973) Male pseudohermaphroditism due to testicular 17-alpha-hydroxysteroid dehydrogenase deficiency. *Journal of Clinical Endocrinology and Metabolism*, **36**, 867–879.

Goebelsmann, U., Zachmann, M., Davajan, V., Israel, R., Mestman, J.H. and Mishell, D.R. (1976) Male pseudohermaphroditism consistent with 17,20-desmolase deficiency. *Gynecologic and Obstetric Investigations*, **7**, 138–156.

Goldberg, E.H. (1988) H-Y antigen and sex determination. *Philosophical Transactions of the Royal Society of London*, Series **322 (1208)**, 73–81.

Goldfoot, D.A. and Van der Werff ten Bosch, J.J. (1975) Mounting behavior of female guinea pigs after prenatal and adult administration of the propionate of testosterone, dihydrotestosterone, and androstanedione. *Hormones and Behavior*, **6**, 139–148.

Goldmann, A.S. and Bongiovanni, A.M. (1967) Induced genital anomalies. *Annals of the New York Academy of Science*, **142**, 755–767.

Gombe, S., Oduor-Okello, D. and Else, J. (1980) The potential of African mammals on new models for research in human reproduction, in *Animal Models in Human Reproduction* (eds M. Serio and L. Martini) Raven Press, New York, pp. 345–358.

Gondos, B. (1985) Development of the reproductive organs. *Annals of Clinical and Laboratory Science*, **15(5)**, 363–373.

Gondos, B. and Hobel, C.J. (1971) Ultrastructure of germ cell development in the human fetal testis. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **119**, 1–20.

Goodfellow, P.N. (1986) The case of the missing H-Y antigen. *Trends in Genetics*, **2**, 87.

Goodlin, B.L. and Sackett, G.P. (1983) Parturition in *Macaca nemestrina*. *American Journal of Primatology*, **4**, 283–307.

Gordon, I., Williams, G.L. and Edwards, J. (1962) The use of serum gonadotrophin (P.M.S.) in the induction of twin-pregnancy in the cow. *Journal of Agricultural Science*, **59**, 153–198.

Gore-Langton, R.E., Tung, P.S. and Fritz, I.B. (1983) The absence of specific interactions in Sertoli-cell secreted proteins with antibodies directed against H-Y antigen. *Cell*, **32**, 289.

Gorski, R.A. (1983) Steroid-induced sexual charac-

teristics in the brain, in *Neuroendocrine Perspectives*, Vol. 2, (eds E.E. Muller, R. M. MacLeod), Elsevier, Amsterdam.

Gorski, R.A. (1984) Critical role for the medial preoptic area in the sexual differentiation of the brain, in *Progress in Brain Research*, Vol. 61 (eds G.J. deVries, J.P.C. deBruin, H.B.M. Uylings and M.A. Corner), Elsevier, Amsterdam, pp. 129–145.

Gorski, R.A. (1985a) Sexual dimorphisms of the brain. *Journal of Animal Science*, **61** Suppl. 3, 38–61.

Gorski, R.A. (1985b) The 13th J.A.F. Stevenson Memorial Lecture. Sexual differentiation of the brain: possible mechanisms and implications. *Canadian Journal of Physiology and Pharmacology*, **63**, 577–594.

Gorski, R.A (1986) Sexual differentiation of the brain: a model for drug-induced alterations of the reproductive system. *Environmental Health Perspectives*, **70**, 163–175.

Gorski, R.A. and Wagner, J.W. (1965) Gonadal activity and sexual differentiation of the hypothalamus. *Endocrinology*, **76**, 226–239.

Gosden, R.G. (1975) Ovarian support of pregnancy in ageing inbred mice. *Journal of Reproduction and Fertility*, **42**, 423–430.

Goulet, L. and Theriault, G. (1987) Association between spontaneous abortion and ergonomic factors. *Scandinavian Journal of Work, Environment and Health*, **13**, 399–403.

Goy, R.W. and McEwen, B.S. (1980) *Sexual Differentiation of the Brain*, MIT Press, Cambridge, Massachusetts.

Goy, R.W., Bercovitch, F.B. and McBrair, M.C. (1988) Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. *Hormones and Behavior*, **22**, 552–571.

Green, J.R. (1984) Placental abnormalities: Placenta previa and abruptio placentae, in *Maternal, Fetal Medicine* (eds R.K. Creasy and R. Resnik), W.B. Saunders, Philadelphia, pp. 539–559.

Greene, R.R. and Burrill, M.W. and Ivy, A.C. (1940) Experimental intersexuality. The effects of estrogens on the antenatal sexual development of the rat. *American Journal of Anatomy*, **67**, 305–345.

Grennan, D.M., McCormick, J.N., Wojtacha, D. and Carty, M. and Behan, W. (1978) Immunological studies of the placenta in systemic lupus erythematosus. *Annals of the Rheumatic Diseases*, **37(2)**, 129–134.

Gribnau, A. and Geijsberts, L. (1981) Developmental stages in the rhesus monkey (*Macaca mulatta*). Vol. 68 *Advances in Anatomy, Embryology and Cell Biology*, Springer-Verlag, New York, pp. 1–84.

Griffin, J.E. (1979) Testicular feminization associated with a thermolabile androgen receptor in cultured human fibroblasts. *Journal of Clinical Investigations*, **64**, 1624–1631.

Griffin, J.E. and Wilson, J.D. (1977) Studies on the pathogenesis of the incomplete forms of androgen resistance in man. *Journal of Clinical Endocrinology and Metabolism*, **45**, 1137–1143.

Griffin, J.E. and Wilson, J.D. (1980) The syndromes of androgen resistance. *New England Journal of Medicine*, **302**, 198–209.

Grimm, E.R. (1962) Psychological investigation of habitual abortion. *Psychosomatic Medicine*, **24**, 369–378.

Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Gabriel, A., Fellous, M. and Weissenbach, J. (1984) Human XX males with Y single-copy DNA fragments. *Nature*, **307**, 172–173.

Gupta, C. and Block, E. (1976) Testosterone-binding protein in reproductive tracts of fetal rats. *Endocrinology*, **99**, 389–399.

Gustavsson, I. (1980) Chromosome aberrations and their influence on the reproductive performance of domestic animals – a review. *Zeitscrift fur Tierzuchtgung und Zuchtgsbiologie*, **97**, 176–195.

Hafez, E.S.E. (1964) Effects of over-crowding in utero on implantation and fetal development in the rabbit. *Journal of Experimental Zoology*, **156**, 269–288.

Hafez, E.S.E. (1967) Reproductive failure in domestic mammals, in *Comparative Aspects of Reproductive Failure* (ed. K. Benirschke), Springer-Verlag, New York, pp. 42–95.

Hafez, E.S.E. (ed.) (1968) *Reproduction in Farm Animals*, 2nd edn, Lea & Febiger, Philadelphia.

Hafez, E.S.E. (1972) Differentiation of mammalian blastocysts, in *Biology of Mammalian Fertilization and Implantation*, (eds K.S. Moghissi and E.S.E. Hafez), Thomas, Springfield, Illinois, pp. 296–342.

Hafez, E.S.E. and Tsutsumi, Y. (1966) Changes in endometrial vascularity during implantation and pregnancy in the rabbit. *American Journal of Anatomy*, **118**, 249–282.

Haines, C.E., Warnick, A.C. and Wallace, H.D. (1959) The effect of two levels of energy intake on reproductive phenomena in Duroc Jersey gilts. *Journal of Animal Science*, **18**, 347–354.

Hahlin, M., Bennegard, B. and Dennefors, B. (1986) Human luteolysis – interaction between hCG and oestradiol 17β in an *in vitro* model. *Human Reproduction*, **1**, 75–79.

Hamberger, L., Dennefors, B., Hamberger, B., Janson, P.O., Nilsson, L., Sjorgen, A. and Wiqvist, N. (1980) Is vascular innervation a prerequisite for PG-induced luteolysis in the human corpus luteum?, in *Advances in Prostaglandin and Thromboxane Research*, (eds B. Samuelsson, P.W. Ramwell and Paoletti), Raven Press, New York, pp. 1365–1368.

Handa, R.J., Corbier. P., Shryne, J.E., Schoonmaker, J.N. and Gorski, R.A. (1985) Differential effects of the perinatal steroid environment on three sexually dimorphic parameters of the rat brain. *Biology of Reproduction*, **32**, 855–864.

Handa, R.J., Connolly, P.B. and Resko, J.A. (1988) Ontogeny of cytosolic androgen receptors in the brain of the fetal rhesus monkey. *Endocrinology*, **122**, 1890–1896.

Hanly, S. (1961) Prenatal mortality in farm animals. *Journal of Reproductive Fertility*, **2**, 182–194.

Hansel, W. (1981) Plasma hormone concentrations associated with early embryo mortality in heifers. *Journal of Reproduction and Fertility*, Suppl. **30**, 231–239.

Hansel, W., Stock, A. and Battista, P.J. (1989) Low molecular weight lipid-soluble luteotrophic factor(s) produced by conceptuses in cows. *Journal of Reproduction and Fertility*, Suppl. **37**, 11–17.

Haour, F., Tell, G. and Sanchez, P. (1976) Mise en évidence et dosage d'une gonadotrophine chorionique chex le rat (rCG). *Comptes Rendus Hebdomadaires des Séances de L'Academie des Sciences (D) Paris*, **282**, 1183–1186.

Harding, P.G.R., Young, A. and Possmayer, F. (1975) The effect of hypoinsulinemia on the fetus (abstract). *Clinical Research*, **23**, 1161.

Harding, R.D., Hulme, M.J., Lunn, S.F., Henderson, C. and Aitken, R.J. (1982) Plasma progesterone levels throughout the ovarian cycle of the common marmoset (*Callithrix jacchus*). *Journal of Medical Primatology*, **11**, 43–51.

Hare, W.C.D. (1976) Intersexuality in the dog. *Canadian Veterinary Journal*, **17**, 7–15.

Harkert, S. and Von-Kreybig, T. (1979) The action of an oestrogen/gestagen combined preparation upon the prenatal development of the rat. *Deutsche Zeitschrift fur Mund-Kiefer-Gesichts Chirurgie*, **3**, 169–176.

Harlan, R. (1826) Description of an hermaphrodite *Orang Outang*, lately living in Philadelphia, **5**, 229–236.

Harlap, S., Shiono, P.H. and Ramcharan, S. (1980) A life table of spontaneous abortions and the effects of age, parity, and other variables, in *Human Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 145–158.

Harlow, C.R., Gems, S., Hodges, J.K. and Hearn, J.P. (1983) The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). *Journal of Zoology (London)*, **201**, 273–282.

Harlow, C.R., Hearn, J.P. and Hodges, J.K. (1984) Ovulation in the marmoset monkey: endocrinology, prediction and detection. *Journal of Endocrinology*, **103**, 17–24.

Hartman, C.G. (1928) The breeding season of the opossum (*Didelphis virginiana*) and the rate of intrauterine and postnatal development. *Journal of Morphology*, **46**, 143–215.

Harwood, J.P., Clayton, R.N., Catt, K.J. (1980) Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. *Endocrinology*, **107**, 407–413.

Haseltine, F. (1983) Sex differentiation: Current concepts, in *Biological Bases of Reproductive and Developmental Medicine* (ed. E.B. Warshaw), Elsevier Biomedical, New York, pp. 179–198.

Hawk, H.W., Tyler, W.J. and Carida, L.E. (1955) Effect of sire and system of mating on estimated embryonic loss. *Journal of Dairy Science*, **38**, 420–427.

Hay, D.L. and Lopata, A. (1990) Factors modifying early HCG production by the human blastocyst *in vitro*. *Assisted Reproduction Technology and Andrology*, **1**, 9–23.

Heap, R.B. and McRae, A. (1988) Implantation, trophoblast signals and maternal responses in *Fetal and Neonatal Development* (ed. C.T. Jones), Perinatology Press, Ithaca.

Heap, R.B., Fleet, I.R. and Hamon, M. (1985) Prostaglandin $F_{2\alpha}$ is transferred from the uterus to the ovary in the sheep by lymphatic and blood vascular pathways. *Journal of Reproduction and Fertility*, **74**, 645–656.

Heap, R.B., Fleet, I.R., Finn, C., Ellis, S.T., Yang, C.B., Whyte, A. and Brigstock, D.R. (1988)

Maternal reactions affecting early embryogenesis and implantation. *Journal of Reproduction and Fertility*, Suppl. **36**, 83–97.

Hearn, J.P. (1975) Immunising marmosets against pregnancy. *Primate Eye*, **4**, 7.

Hearn, J.P. (1976) Immunisation against pregnancy. *Proceedings of the Royal Society of London* **B 195**, 149–160.

Hearn, J.P. (1978) Immunological suppression of fertility in primates, in *Recent Advances in Primatology*, Vol. 4 (eds D.J. Chivers and E.H.R. Ford), Academic Press, London, pp. 141–143.

Hearn, J.P. (1979) Immunological interference with the maternal recognition of pregnancy in primates, in *Maternal Recognition of Pregnancy* (Ciba Foundation Syposium 64), Elsevier, Amsterdam, pp. 353–375.

Hearn, J.P. (1980) Endocrinology and timing of implantation in the marmoset monkey, *Callithrix jacchus*. *Progress in Reproductive Biology*, **7**, 262–244.

Hearn, J.P. (1983) The common marmoset (*Callithrix jacchus*), in *Reproduction in New World Primates* (ed. J.P. Hearn), MTP Press, Lancaster, pp. 181–215.

Hearn, J.P. (1984) Lactation and reproduction in non-human primates. *Symposia of the Zoological Society of London*, **51**, 327–335.

Hearn, J.P. (1986) The embryo–maternal dialogue during early pregnancy in primates. *Journal of Reproduction and Fertility*, **76**, 809–819.

Hearn, J.P. and Summers, P.M. (1986) Experimental manipulation of embryo implantation in the marmoset monkey and exotic equids. *Theriogenology*, **25**, 3–11.

Hearn, J.P. and Webley, G.E. (1987) Regulation of the corpus luteum of early pregnancy in the marmoset monkey: local interactions of luteotrophic and luteolytic hormones *in vivo* and their effects on the secretion of progesterone. *Journal of Endocrinology*, **114**, 231–239.

Hearn, J.P., Short, R.V. and Baird, D.T. (1977) Evolution of the luteotrophic control of the mammalian corpus luteum, in *Reproduction and Evolution* (eds J.H. Calaby and C.H. Tyndale-Biscoes), Australian Academy of Science, Canberra, pp. 255–263.

Hearn, J.P., Summers, P.M. and Webley, G.E. (1987) Intraembryonic and luteal effects of chorionic gonadotrophin during the peri-implantation period in a primate, *Callithrix jacchus*, in *Highlights on Endocrinology* (eds C. Christiansen and B.D. Riis), Proceedings of the 1st European Congress of Endocrinology, Norhaven Bogtrykken, Copenhagen, pp. 281–286.

Hearn, J.P., Hodges, J.K. and Gems, S. (1988a) Early secretion of chorionic gonadotrophin by marmoset embryos *in vivo* and *in vitro*. *Journal of Endocrinology*, **119**, 249–255.

Hearn, J.P., Gidley-Baird, A.A., Hodges, J.K., Summers, P.M. and Webley, G.E. (1988b) Embryonic signals during the peri-implantation period in primates. *Journal of Reproduction and Fertility*, Suppl. **36**, 49–58.

Hearn, J.P., Webley, G.E. and Gidley-Baird, A.A. (1988c) Embryo implantation in Primates, in *Implantation, Biological and Clinical Aspects* (eds M. Chapman, G. Grudzinskas and T. Chard), Springer-Verlag, London, pp. 3–13.

Hearn, J.P., Webley, G.E. and Gidley-Baird, A.A. (1991) Chorionic gonadotrophin and embryo–maternal recognition during the peri-implantation period in primates. *Journal of Reproduction and Fertility*, **92**, 497–509.

Hearnshaw, H., Restall, B.J. and Gleeson, A.R. (1973) Observations on the luteolytic effects of prostaglandin F_2 during the oestrous cycle and early pregnancy in the ewe. *Journal of Reproduction and Fertility*, **32**, 322–323.

Heger, W., Merker, H.J. and Neubert, D. (1988) Frequency of prenatal loss in marmoset monkeys (*Callithrix jacchus*) in *Non-Human Primates – Developmental Biology and Toxicology* (ed. A.G. Hendrickx), Ueberreuter Wissenschaft, Wien, Berlin, pp. 129–140.

Helmer, S.D., Hansen, P.J., Thatcherm, W.W., Johnson, J.W. and Bazer, F.W. (1989a) Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an anti-luteolytic effect to extend corpus luteum life-span in cyclic cattle. *Journal of Reproduction and Fertility*, **87**, 89–101.

Helmer, S.D., Gross, T.S., Newton, G.R., Hansen, P.J. and Thatcher, W.W. (1989b) Bovine trophoblast protein-1 complex alters endometrial protein and prostaglandin secretion and induces an intracellular inhibitor of prostaglandin synthesis *in vitro*. *Journal of Reproduction and Fertility*, **87**, 421–430.

Hemminki, K. and Vineis, P. (1985) Extrapolation of the evidence on teratogenicity of chemicals between humans and experimental animals: chemicals other than drugs. *Teratog. Carcinog. Mutag.* **5**, 251–318.

Hendrickx, A.G. (1966) Teratogenicity findings in

a baboon colony, in *F.D.A. Conference on Nonhuman Primate Toxicology* (ed. C.O. Miller), US Government Printing Office, Washington, D.C., pp. 120–123.

Hendrickx, A.G. (1971) *Embryology of the Baboon*, The University of Chicago Press, Chicago

Hendrickx, A.G. and Binkerd, P.E. (1980) Fetal deaths in nonhuman primates, in *Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 45–69.

Hendrickx, A.G. and Binkerd, P.E. (1990) Nonhuman primates and teratological research. *Journal of Medical Primatology*, **19**, 81–108.

Hendrickx, A.G. and Enders, A.C. (1980) Implantation in non-human primates. II. Endocrinology, in *Non-human Models for Study of Human Reproduction* (ed. T.C. Anand Kumar), Karger, Basle, pp. 109–116.

Hendrickx, A.G. and Houston, M.C. (1970) Gestation and prenatal development, in *Reproduction and Breeding Techniques for Laboratory Animals* (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 157–176.

Hendrickx, A.G., Korte, R., Leuschner, F., Neumann, B.W., Poggel, A., Binkerd, P., Prahalada, S. and Gunzel, P. (1987a) Embryotoxicity of sex steroidal hormones in nonhuman primates. II. Hydroxyprogesterone caproate. Estradiol valerate. *Teratology*, **35**, 129–136.

Hendrickx, A.G., Korte, R., Leuschner, F., Neumann, B.W., Prahalada, S., Poggel, A., Binkerd, P.E. and Gunzel, P. (1987b) Embryotoxicity of sex steroidal hormone combination in nonhuman primates. I. Norethisterone acetate and Ethinyglestradiol and Progesterone and Estradiol Benzoate (*Macaca mulatta, Macaca fascicularis*, and *Papio cyrocephalus*). *Teratology*, **35**, 119–127.

Herbst, A., Ulfelder, H. and Poskanzer, D. (1971) Adenocarcinoma of the vagina: association of maternal stilboestrol therapy with tumor appearance in young women. *New England Journal of Medicine*, **284**, 878–881.

Herbst, E.W., Fredge, K., Frank, F., Winking, H. and Gropp, A. (1978) Cystological identification of two X-chromosome types in the wood lemming (*Myopus schisticolor*). *Chromosomes*, **69**, 185–191.

Hertig, A.T. (1967) The overall problem in man, in *Comparative Aspects of Reproductive Failure* (ed. K. Benirschke), Springer-Verlag, New York, pp. 11–41.

Hertig, A.T., Rock, J. and Adams, E.C. (1956) A description of 34 human ova within the first 17 days of development. *American Journal of Anatomy*, **98**, 435–494.

Hertig, A.T., Rock, J., Adams, E.C. and Menkin, M.C. (1959) Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility: A study of biologic wastage in early human pregnancy. *Pediatrics*, **23**, 202–211.

Hertig, A.T., King, N.W. and Mackey, J. (1971) Spontaneous abortion in wild-caught rhesus monkeys, *Macaca mulatta*. *Laboratory Animal Science*, **21**, 510–519.

Heuser, C.H. (1940) Chimpanzee ovum in early stages of implantation (about 10½ days). *Journal of Morphology*, **66**, 155–173.

Heuser, C.H. and Streeter, G.L. (1941) Development of the macaque embryo. *Contributions to Embryology*. Carnegie Institute of Washington (179–186) **29**, 15–56.

Heyman, Y., Camous, S., Fevre, J., Meziou, W. and Martal, J. (1984) Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. *Journal of Reproduction and Fertility*, **70**, 533–540.

Heyner, S., Smith, R.M. and Schultz, G.A. (1989) Temporally regulated expression of insulin and insulin-like growth factors and their receptors in early mammalian development. *Bioessays*, **11**, 171–176.

Hickey, G.J. and Hansel, W. (1987) *In-vitro* synthesis of a low molecular weight lipid-soluble luteotrophic factor by conceptuses of cow at day 13–18 of pregnancy. *Journal of Reproduction and Fertility*, **80**, 569–576.

Hicks, H.T. and French, H. (1905) Typhoid fever and pregnancy with special reference to foetal infection. *Lancet*, **1**, 1491–1493.

Hill, D.E. (1976) Insulin and fetal growth, in *Diabetes and Other Endocrine Disorders During Pregnancy and in the Newborn* (eds M.I. New and R.H. Fiser), Alan R. Liss, New York, pp. 127–139.

Hill, J.P. (1932) The developmental history of the primates. *Philosophical Transitions of the Royal Society of London (Biology)*, **221**, 45–178.

Hillier, S.G. and Cameron, E.H.D. (1976) Physiological effects of immunity against steroid hormones in the rat, in *Physiological Effects of Immunity Against Reproductive Hormones* (eds R.G. Edwards and M.H. Johnson), Cambridge University Press, pp. 91–120.

Hirobe, S., He, W.W., Lee, M.M. and Donahoe,

P.K. (1992) Mullerian inhibiting substance messenger ribonucleic acid expression in granulosa and Sertoli cells coincides with their mitotic activity. *Endocrinology*, **131**, 854–862.

Ho, C. and Aterman, K. (1970) Infection of the fetus by *Candida* in a spontaneous abortion. *American Journal of Obstetrics and Gynecology*, **106**, 705–710.

Hoar, R.M. (1978) Guinea pigs (developmental abnormalities), in *Pathology of Laboratory Animals*, Vol 2 (eds K. Benirschke, F.M. Garner M. and T.C. Jones), Springer-Verlag, New York, pp. 1860–1866.

Hodges, J.K. Green, D.I., Cottingham, P.G., Sauer, M.J., Edwards, C. and Lightman, S.L. (1988) Induction of luteal regression in the marmoset monkey (*Callithrix jacchus*) by a gonadotrophin-releasing hormone antagonist and the effects on subsequent follicular development. *Journal of Reproduction and Fertility*, **82**, 743–752.

Hofsaess, F.R. and Meachan, T.N. (1971) Chromosome abnormalities of early rabbit embryos. *Journal of Experimental Zoology*, **177**, 9–12.

Hogan, M.D., Newbold, R.R. and McLachlan, J.A. (1987) Extrapolation of teratogenic responses observed in laboratory animals to humans: DES as an illustrative example in *Developmental Toxicology: Mechanisms and Risks* (eds J.A. McLachlan, R.M. Pratt and C.L. Markert), Banbury Report Vol. **26**, Cold Spring Harbor Symposium, pp. 257–269.

Hollander, W.F. and Strong, L.C. (1950) Intrauterine mortality and placental fusions in the mouse. *Journal of Experimental Zoology*, **115**, 131–149.

Holst, P.A. and Phemister, R.D. (1971) The prenatal development of the dog: Preimplantation events. *Biology of Reproduction*, **5**, 194–206.

Holter, J.A. and Andrews, J.J. (1979) Evaluation of current diagnostic methods for causes of abortions. *American Association of Veterinary Laboratory Diagnostics – 22nd Annual Proceedings*, 85–94.

Honnebier, W.J. and Swaab, D.R. (1974) Influence of a-melanocyte-stimulating hormone (a-MSH), growth hormone (GH) and fetal brain extracts on intrauterine growth of fetus and placenta in the rat. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 439–447.

Hood, R.D. (1972) Effects of sodium arsenate on fetal development. *Bulletin of Environmental Contamination and Toxicology*, **7**, 216–222.

Horowitz, M. (1967) The anovular corpus luteum in the golden hamster (*Mesocricetus auratus Waterhouse*) and comparisons with the normal corpus luteum and follicle of atresia. *Acta Anatomica*, **66**, 199–225.

Horton, E.W. and Poyser, N.L. (1976) Uterine luteolytic hormone: a physiological role for prostaglandin $F_{2\alpha}$. *Physiological Reviews*, **56**, 595–651.

Houmard, B.S., Guan, Z., Stokes, B.T. and Ottobre, J.S. (1992) Activation of the phosphatidylinositol pathway in the primate corpus luteum by prostaglandin $F_{2\alpha}$. *Endocrinology*, **131**, 743–748.

Howarth Jr., B. and Hawk, H.W. (1968) Effect of hydrocortisone on embryonic survival in sheep. *Journal of Animal Science*, **27**, 117–121.

Hsu, C. (1948) Influence of temperature on development of rat embryos. *Anatomical Record*, **100**, 79–90.

Hubbert, W.T., Booth, G.D. and Bolton, W.D. (1973) Bovine abortions in five Northeastern states, 1960–1970: Evaluation of diagnostic laboratory data. *Cornell Veterinarian*, **63**, 291–316.

Hudak, A., Rodics, K., Stuber, I., Ungvary, G., Krasznai, G., Szomolanyi, I. and Csonka, A. (1977) The effects of toluene inhalation on pregnant cfy rats and their offspring. *Munka-Uzemegeszegugyi Intez.*, Hungary, Munkavedelem, **23**, Suppl, 25–30.

Humphrey, K.W. and Martin, L. (1969) The effects of oestrogens and antioestrogens on ovum transport in the laboratory mouse. *Australian Journal of Biological Science*, **21**, 1239–1248.

Hunter, R.H.F. (1974) Chronological and cytological details of fertilization and early embryonic developments in the domestic pig, *Sus scrofa*. *Anatomical Record*, **178**, 169–185.

Iannaccone, P.M., Bossert, N.L. and Connelly, C.S. (1987) Disruption of embryonic and fetal development due to preimplantation chemical insults: A critical review. *Obstetrics and Gynecology*, **157**, 476–484.

Imakawa, K., Anthony, R.V., Kazemi, M., Marotti, K.R., Polites, H.G. and Roberts, R.M. (1987) Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. *Nature* **330**, 377–379.

Imakawa, K., Hansen, T.R., Malathy, P.V., Anthony, R.V., Polites, H.G., Marotti, K.R., Roberts, R.M. (1989) Molecular cloning and characterisation of cDNAs corresponding to bovine trophoblast protein-1: a comparison with

ovine trophoblast protein-1 and bovine interferon $_{II}$. *Molecular Endocrinology*, **3**, 127–139.

Imperato-McGinley, J., Guerrero, L., Gautier, T. and Peterson, R.E. (1974) Steroid 5-alpha-reductase deficiency in man: An inherited form of male pseudohermaphroditism. *Science*, **186**, 1213–1215.

Ingram, D.L., Mandl, A.M. and Zuckerman, S. (1958) The influence of age on litter size. *Journal of Endocrinology*, **17**, 280–285.

Jacobs, P.A. (1982) Pregnancy losses and birth defects, in *Reproduction in Mammals, Book 2, Embryonic and Fetal Development* (eds C.R. Austin and R.V. Short), Cambridge University Press, Cambridge, pp. 142–158.

Jacquot, R. (1959) Recherches surle contrôle endocrinien de l'accumulation de glyco géne dans le foie chez le foetus de rat. *Journal of Physiology (Paris)*, **51**, 655–691.

Jean, C. (1966) Malformations of the genitalia of adult female mice after injection of oestrogens to their mothers during pregnancy. *Compte Rendu de la Société de Biologie de Clermont-Ferrand*, **160**, 976–981.

Jean, C. (1971) Analyses des malformations mammaires due nouveau-né provoquees par l'injection d'oestrogenes a la mere gravide chez le rat et la souris. *Archives d'Anatomie Microscopique*, **60,** 147–168.

Jean, C. and Jean, C. (1970) Influence des oestrogens injectés a la mere gravide sur la mortalité du foetus et du nouveau-né. *Compte Rendu des séances de la Société de Biologie de Clermont-Ferrand*, **164**, 779–783.

Jimenez de Asva, L.S., Carr, B., Clingon, D. and Rudland, P. (1977) Specific glucocorticoid, inhibition of growth promoting effects of prostaglandin F and 3T3 cells. *Nature*, **265**, 450–452.

Jirasek, J.E. (1971) *Development of the Genital System and Male Pseudohermaphroditism*, Johns Hopkins Press, Baltimore.

Jirasek, J.E. (1976) Principles of reproductive endocrinology, in *Disorders of Sexual Differentiation* (ed. J.L. Simpson), Academic Press, New York pp. 52–111.

Jirasek, J.E. (1977) Morphogenesis and malformation of the genital system, in *Birth Defects*, Vol. 13 (eds R.J. Blandau and D. Bergsman), Alan R. Liss, New York, pp. 13–39.

Johnson, D.C. (1966) An anovulatory bilateral hermaphrodite rat. *Nature*, **210**, 1287–1288.

Johnson, L.D. (1984) The effects of prenatal diethylstilbestrol exposure on the genital tracts of fetuses and neonates (*Cebus apella*, *Macaca mulatta* and *Homo sapiens*), in *One Medicine* (eds O.A. Ryder and M.L. Byrd), Springer-Verlag, Berlin, pp. 197–216.

Johnson, L.D., Palmer, A.E., King, N.W. Jr. and Hertig, A.T. (1981) Vaginal adenosis in *Cebus apella* monkeys exposed to DES in utero. *Obstetrics and Gynecology*, **57**, 629.

Johnson, M.H., Chisholm, J.C., Fleming, A. and Houliston, E. (1986) A role for cytoplasmic determinants in the development of the mouse early embryo. *Journal of Embryology and Experimental Morphology*, **97** Suppl, 97–121.

Johnston, E.F., Zeller, J.H. and Cantwell, G. (1958) Sex anomalies in swine. *Journal of Heredity*, **49**, 255–261.

Jones, E.C. and Krohn, P.L. (1961) The relationships between age, numbers of oocytes and fertility in virgin and multiparous mice. *Journal of Endocrinology*, **21**, 469–95.

Jones, G.E.S. and Delfs, E. (1951) Endocrine patterns in term pregnancies following abortion. *Journal of the American Medical Association*, **146**, 1212–1218.

Jones, G.S. and Pourmand, K. (1962) An evaluation of etiologic factors and therapy in 555 private patients with primary infertility. *Fertility and Sterility*, **13**, 398–410.

Jones, P.R.M., Peters, J. and Bagnall, K.M. (1986) Anthropometric measures of fetal growth, in *Human Growth*, Vol. l, Developmental Biology Prenatal Growth (eds F. Falkner and J.M. Tanner), Plenum Press, New York, pp. 255–274.

Jones, W.R. (1976) Immunologic aspects of infertility, in *Immunology of Human Reproduction* (eds J.S. Scott and W.R. Jones), Academic Press, London, pp. 375–413.

Josso, N. (1970) Action de la testostérone sur le canal de Wolff du foetus de rat en culture organotypique. *Archives d'Anatomie Microscopique*, **59**, 37–50.

Josso, N. (1981) Differentiation of the genital tract: Stimulators and inhibitors, in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 165–203.

Josso, N,. Picard, J.Y., and Tran, D. (1977) The anti-mullerian hormone. *Recent Progress in Hormone Research*, **33**, 117–l60.

Jost, A. (1947) Recherches sur la différenciation sexuelle de l'embryon de lapid. III. Rôle des gonades foetales dans la différenciation sexuelle

somatique. *Archives d'Anatomie Microscopique*, **36**, 371–315.

Jost, A. (1953) Problems of fetal endocrinology: The gonadal and hypophyseal hormones. *Recent Progress in Hormone Research*, **8**, 379–418.

Jost, A. (1970) Hormonal factors in the sex differentiation of the mammalian foetus. *Philosophical Transactions of the Royal Society of London*, B 259, 119–131.

Jost, A. (1972) Données preliminaires sur les stades initiaux de la différenciation du testicule chez le rat. *Archives d'Anatomie Microscopique et de Morphologie Experimentale*, **6l**, 415–438.

Jost, A. (1979) Fetal hormones and fetal growth. *Contributions to Gynecology and Obstetrics*, **5**, 1–20.

Jost, A. (1985) Initial stages of gonadal development. Theories and methods. *Archives D Anatomie Microscopique et de Morphologie Experimentale*, **74(1)**, 39–41.

Jost A. and Magre, S. (1988) Control mechanisms of testicular differentiation. *Philosophical Transactions of the Royal Society of London*. Series 322(1208), 55–61.

Jost, A., Vigier, B., Prepin, J. and Perchellet, J.P. (1973) Studies on sex differentiation in mammals. *Recent Progress in Hormone Research*, **29**, 1–41.

Jost, A., Perlman, S. and Magre, S. (1985) The initial stages of testicular differentiation in the rabbit fetus. *Archives D Anatomie Microscopique et de Morphologie Experimentale*, **74(1)**, 69–75.

Jung, H. and Peters, A. (1967) The action of various gestagens upon the development of the foetus and the incidence of deaths in animal experiments. *Archive fur Gynaekologie*, **204**, 68–77.

Jung, I. and Baulieu, E.E. (1972) Testosterone cytosol 'receptor' in the rat levator ani muscle. *Nature New Biology*, **237**, 24–26.

Kaij, L., Malmquist. A. and Nilsson, A. (1969) Psychiatric aspects of spontaneous abortion. II. The importance of bereavement attachment and neurosis in early life. *Journal of Psychosomatic Research*, **13**, 53–59.

Kalter, H. (1968) *Teratology of the Central Nervous System*, University of Chicago Press, Chicago, pp. 483.

Kalter, H. (1975) Prenatal epidemiology of spontaneous cleft lip and palate, open eyelid, and embryonic death in A/J mice. *Teratology*, **12**, 245–258.

Kalter, H. (1980) The relation between congenital malformation and prenatal mortality in *Human* experimental animals, in *Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 29–44.

Kankaanpaa, J.T.J., Elovaara, E., Hemminki, K. and Vainio, H. (1980) The effect of maternally inhaled styrene on embryonal and foetal development in mice and Chinese hamsters. *Acta Pharmacologica Toxicologica*, **47**, 127–129.

Kato, T., Yakushiji, M., Tuda, H., Arima, A., Takahashi, K., Shimomura, M., Miyahara, M., Adachi, M. and Tashiro, Y. (1972) Polychlorobiphenyls (PCB) induced fetopathy. II. Experimental studies. Possible placental transfer of polychlorobiphenyls in rats. *Kurume Medical Journal*, **19**, 53–59.

Katz, A., Lancet, M., Skornik, J., Chemke, J., Mogilner, B.M. and Klinberg, M. (1985) Teratogenicity of progestogens given during the first trimester of pregnancy. *Obstetrics and Gynecology*, **65**, 775–780.

Kellokumpu-Lehtinen, P., Santti, R. and Pelliniemi, L.J. (1979) Early cytodifferentiation of human prostatic urethra and Leydig cells. *Anatomical Record*, **194**, 429–444.

Kelly, J.V. and Iffy, L. (1981) Placenta previa, in *Principles and Practice of Obstetrics and Perinatology*, Vol 2 (eds L. Iffy and H.A. Kaminetsky), John Wiley & Sons, New York, pp. 1105–1120.

Kennedy, G.L., Arnold, D.W. and Calandra, J.C. (1975) Teratogenic evaluation of lead compounds in mice and rats. *Food and Cosmetics Toxicology*, **13**, 629–632.

Kent Jr., H.A. (1962) Polyovular follicles and multinucleate ova in the ovaries of young hamsters. *Anatomical Record*, **143**, 345–349.

Khan-Dawood, F.S. (1986) Localization of oxytocin and neurophysins in baboon (*Papio anubis*) corpus luteum by immunocytochemistry. *Acta Endocrinologica* (Copenhagen), **113**, 570–575.

Khan-Dawood, F.S., Marut, E.L. and Dawood, M.Y. (1984) Oxytocin in the corpus luteum of the cynomologus monkey (*Macaca fascicularis*). *Endocrinology*, **115**, 570–574.

Khera, K.S. (1973) Teratogenic effects of methylmercury in the cat: Note on the use of this species as a model for teratogenicity studies. *Teratology*, **8**, 293–304.

Khera, K.S. (1984) Adverse effects in humans and animals of prenatal exposure to selected therapeutic drugs and estimation of embryo–fetal sensitivity of animals for human risk assessment. A review, in *Issues and Reviews in*

Teratology (ed. H. Kalter), Plenum Press, New York, pp. 399–507.

Kiel-Metzger, K., Erickson, R.P. (1984) Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 of mice. *Nature*, **310**, 579–581.

Kiel-Metzger, K., Warren, G., Wilson, G.N. and Erickson, R.P. (1985) Evidence that the human Y chromosome does not contain clustered DNA sequences (Bkm) associated with heterogametic sex determination in other vertebrates. *New England Journal of Medicine*, **313**, 242–245.

Kihlstrom, J.E. (1972) Period of gestation and body weight in some placental mammals. *Comparative Biochemistry and Physiology*, **43A**, 673–679.

Kikuchi, Y., Hitotsumachi, S., Nomura, M. and Nakaguchi, T. (1977) A true hermaphrodite rat (*Rattus norvegicus*) with 41,X/42,XYq-comple ment. *Japanese Journal of Genetics*, **52**, 73–79.

Kimball, A.C., Kean, B.H. and Fuchs, F. (1971) The role of toxoplasmosis in abortion. *American Journal of Obstetrics and Gynecology*, **111**, 219–226.

Kimmel, C.A. and LaBorde, J.B. (1979) Teratogenic potential of ethylene oxide. *Teratology*, **19**, 34A–35A.

Kimmel, G.L., Kimmel, C.A., Francis, E.Z. (eds) (1986) Evaluation of Maternal and Developmental Toxicity. *Teratogenesis, Carcinogenesis, and Mutagenesis*, **7**, 203–338.

Kimura, T. (1975) Persistent vaginal cornification in mice treated with estrogen prenatally. *Endocrinologica Japonica*, **22**, 497–502.

King, C.R. and Pernoll, M.L. (1984) Sexual differentiation. *Obstetrics and Gynaecology Annual*, **13**, 1–33.

King, J.W.B. and Young, G.B. (1957) Maternal influences on litter size in pigs. *Journal of Agricultural Science*, **48**, 457–463.

King, N.W. (1978) The reproductive tract, in *Pathology of Laboratory Animals* (eds K. Benirschke, F.M. Garnes and T.C. Jones), Springer-Verlag, Berlin, pp. 509–580.

King, N.W. and Garvin, C.H. (1964) Bilateral hermaphroditism in a dog. *Journal of the American Veterinary Medicine Association*, **145**, 997–1001.

Kinsky, I. (1979) Bildung des somatischen Gonadenblastems durch degenerierende Urnierenanteile des Kaninchens. *Verhandlungen Anatomischen Gessellschaft* (Innsbruck), **73**, 403–406.

Kirkland, R.T., Kirkland, J.L., Johnson, C., Horning, M., Librik, L. and Clayton, G.W. (1973) Congenital lipoid adrenal hyperplasia in an eight-year-old phenotypic female. *Journal of Clinical Endocrinology and Metabolism*, **36**, 488–496.

Kline, J. and Stein, Z. (1985) Very early pregnancy, in *Reproductive Toxicology* (ed. R.L. Dixon), Raven Press, New York, pp. 251–265.

Kline, J. and Stein, Z. (1987) Epidemiology of chromosomal anomalies in spontaneous abortion: prevalence, manifestation and determinants in *Spontaneous and Recurrent Abortion* (eds M.J. Bennett and D.K. Edmonds), Blackwell Scientific Publications, Oxford, pp. 29–49.

Knickerbocker, J.J., Thatcher, W.W., Bazer, F.W., Drost, M., Barron, D.H., Fincher, K.B. and Roberts, R.M. (1986) Proteins secreted by Day-16 to Day-18 bovine conceptuses extend corpus luteum function in cows. *Journal of Reproduction and Fertility*, **77**, 381–391.

Knobil, E. (1973) On the regulation of the primate corpus luteaum. *Biology of Reproduction*, **8**, 246–258.

Knox, G.E. (1978) Influence of infection of fetal growth and development. *Journal of Reproductive Medicine*, **21**, 352–358.

Knudsen, O. (1956) Some aspects of chromosomes in andrological diagnosis. *Proceedings of the IIIrd International Congress of Animal Reproduction*, Section I.

Koch, W. (1961) Intersexualitat bei Saugetieren, in *Die Intersexualitat* (ed. C. Overzier), Thieme, Stuttgart, pp. 37–49.

Kochenour, N.K. (1987) Other causes of fetal death. *Clinical Obstetrics and Gynecology*, **30**, 312–321.

Koford, C.B. (1965) Population dynamics of rhesus monkeys on Cayo Santiago, in *Primate Behavior* (ed. I. Devore), Holt, Rhinehart & Winston, New York, pp. 160–174.

Kohrs, M.B., Harper, A.E. and Kerr, G.R. (1976) Effects of a low-protein diet during pregnancy of the rhesus monkey. I. Reproductive efficiency. *American Journal of Clinical Nutrition*, **29**, 136–145.

Komlos, L., Zamir, R., Joshua, H, and Halbrecht, I. (1977) Common HLA antigens in couples with repeated abortions. *Clinical Immunology and Immunopathology*, 7 **(3)**, 330–335.

Kretchmer, N., Schumacher, L.B. and Silliman, K. (1989) Biological factors affecting intrauterine growth. *Seminars in Pathology*, **13**, 169–179.

Kriel, R.L., Gates, G.A., Wulff, H., Powell, N., Poland, J.D. and Chin, T.D.Y. (1970) Cytomegalovirus isolations associated with

pregnancy wastage. *American Journal of Obstetrics and Gynecology*, **106**, 885–892.

Kromka, M. and Hoar, R.M. (1973) Use of guinea pigs in teratological testing. *Teratology*, **7**, A21–23.

Kronick, J.B. (1976) Teratogenic effects of ethyl alcohol administered to pregnant mice. *American Journal of Obstetrics and Gynecology*, **124**, 676–680.

Kruger, H. and Arias-Stella, J. (1970) The placenta and the newborn at high altitudes. *American Journal of Obstetrics and Gynecology*, **106**, 586–591.

Kundsin, R.B., Driscoll, S.G. and Ming, P.L. (1967) Strain of *Mycoplasma* associated with human reproductive failure. *Science*, **157**, 1573–1574.

Kundsin, R.B., Rowell, T., Shepard, M.C., Parreno, A. and Lunceford, C.D. (1975) T-strain mycoplasmas and reproductive failure in monkeys. *Laboratory Animal Science*, **25**, 221–224.

Lachtan Hay, D. and Lopata, A. (1988) Chorionic gonadotrophin secretion by human embryos *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **67**, 1322–1324.

Laherty, R.F., Rotten, D., Yamamoto, M. and Jaffe, R.B. (1985) Effects of oestradiol and prolactin on progesterone production by rhesus monkey luteal cells *in vitro*. *Acta Endocrinologica*, **108**, 266–272.

Laing, J.A. (1949) Infertility in cattle associated with death of ova at early stages after fertilization. *Journal of Comparative Pathology*, **59**, 97–108.

Langman, J. (1975) *Medical Embryology*, Williams & Wilkins, Baltimore and London.

Laron, Z. and Pertzelan (1969) Somatotrophin antenatal and perinatal growth and development. *Lancet*, **1**, 680–681.

Lauritsen, J.G., Jorgensen, J. and Kissmeyer-Nielsen, K.F. (1976) Significance of HLA and blood-group incompatibility in spontaneous abortion. *Clinical Genetics*, **9**, 575–582.

Lawrence, W.D., Whitaker, D., Sugimura, H., Cunha, G.R., Dickersin, G.R. and Robboy, S.J. (1992) An ultrastructural study of the developing urogenital tract in early human fetuses. *American Journal of Obstetrics and Gynecology*, **167**, 185–193.

Learner, L.J., DePhillips, M., Yiacas, E., Brennan, D. and Borman, A. (1962) A comparison of the acetophenone derivative of 16, 17- dihydroxyprogesterone with other progestational steroids for masculinization of the rat fetus. *Endocrinology*, **71**, 448–451.

Lee, P.C. (1987) Nutrition, fertility and maternal investment in primates. *Journal of Zoology* (London), **213**, 409–422.

Leese, H.J. (1988) The formation and function of oviduct fluid. *Journal of Reproduction and Fertility*, **82**, 843–856.

de Lemos, R.A. (1976) Glucocorticoid effect: Organ development in monkeys, in *Lung Maturation and the Prevention of Hyaline Membrane Disease* (ed. T.D. Moore), Report of the 70th Ross Conference on Pediatric Research, Ross Laboratories Columbus, Ohio, pp. 77–80.

Lenton, E.A. and Woodward, A.J. (1988) The endocrinology of conception cycles and implantation in women. *Journal of Reproduction and Fertility*, Suppl. 36, 1–15.

Lenton, E.A., Osborn, J., Colman, C. and Fothergill, D. (1988) Premenstrual pregnancy loss. *Annals of the New York Academy of Science*, **541**, 498–509.

Leridon, H. (1977) *Human Fertility*, University of Chicago Press, Chicago, pp. 202.

Levine, L.S., Lieber, E., Pang, S. and New, M.I. (1980) Male pseudohermaphroditism due to 17-ketosteroid reductase deficiency diagnosed in the newborn period. *Pediatric Research*, **14**, 480.

Levine, M.M., Edsall, G. and Bruce-Chwatt, L.J. (1974) Live virus vaccines in pregnancy: risks and recommendations. *Lancet*, **2**, 34–38.

Liggins, G.C. (1974) The influence of the fetal hypothalamus and pituitary on growth, in *Size at Birth* (eds K. Elliot and J. Knight) (Ciba Foundation Symposia, Vol. 27), Elsevier-Excerpta Medica, Amsterdam, pp. 165–184.

Liggins, G.C. (1982) The fetus and birth, in *Reproduction in Mammals. 2. Embryonic and Fetal Development* (eds C.R. Austin and R.V. Short), Cambridge University Press, Cambridge, pp. 114–141.

Loke, Y.W. and Whyte, A. (eds) (1983) *Biology of Trophoblast*, Elsevier, Amsterdam.

London, W.T., Curfman, B. and Sever, J.L. (1971) Mumps infection of the rhesus monkey fetus. *Teratology*, **4**, 234–235.

Long, S.E. and Williams, C. (1978) Chromosomal abnormalities in ova and zygotes from normal ewes mated to normal rams. *Veterinary Record*, 102**(7)**, 153.

Lowrey, G.H. (1978) *Growth and Development of Children*, 7th edn, Year Book Medical Publishers, Chicago.

Lu, C.-C., Matsumoto, N. and Iijima, S. (1979) Teratogenic effects of nickel chloride on embry-

onic mice and its transfer to embryonic mice. *Teratology*, **19**, 137–142.

Luckett, W.P. (1978) Origin and differentiation of the yolk sac and extraembryonic mesoderm in presomite human and rhesus monkey embryos. *American Journal of Anatomy*, **152**, 59–98.

Lukaszewska, J. and Hansel, W. (1980) Corpus luteum maintenance during early pregnancy in the cow. *Journal of Reproduction and Fertility*, **59**, 485–493.

Lushbaugh, C.C., Humason, G. and Gengozian, N. (1969) Intrauterine death from congenital Chagas' disease in laboratory bred marmosets (*Saguinus fuscicollis lagonotus*). *American Journal of Tropical Medicine and Hygiene*, **18**, 662–665.

Lynch, F.W. (1932) Dermatologic conditions of the fetus, with particular reference to variola and vaccinia. *Archives of Dermatological Research* (Berlin), **26**, 997–1019.

Lyon, M.F. (1969) A true hermaphrodite mouse presumed to be an XO/XX mosaic. *Cytogenetics*, **8**, 326–331.

Lyon, M.F. and Hawkes, S.G. (1970) X linked gene for testicular feminization in the mouse. *Nature*, **227**, 1217–1219.

Lyon, M.F., Cattanach, B.M. and Charlton, H.M. (1981) Genes affecting sex differentiation in mammals, in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 329–386.

McAnulty, J.H., Metcalfe, T. and Veland, K. (1982) Cardiovascular disease, in *Medical Complications During Pregnancy* (eds G.N. Burrow and T.F. Ferris), W B Saunders, Philadelphia, pp. 145–146.

McCance, R.A. and Widdowson, E.M. (1986) Glimpses of comparative growth and development, in *Human Growth*, Vol. 1. *Developmental Biology Prenatal Growth* (eds F. Falkner and J.M. Tanner), Plenum Press, New York, pp. 133–15l.

McClain, R.M. and Becker, B.A. (1972) Effects of organolead compounds on rat embryonic and fetal development. *Toxicology and Applied Pharmacology*, **21**, 265–274.

McClain, R.M. and Becker, B.A. (1975) Teratogenicity, fetal toxicity and placental transfer of lead nitrate in rats. *Toxicology and Applied Pharmacology*, **31**, 72–82.

McClure, T.J. (1959) Temporary nutritional stress and infertility in female mice. *Journal of Physiology*, **147**, 221–225.

McClure, T.J. (1961) Uterine pathology of temporarily-fasted pregnant mice. *Journal of Comparative Pathology and Therapeutics*, **71**, 16–19.

McCracken, J.A., Glew, M.B. and Scaramuzzi, R.J. (1970) Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. *Journal of Clinical Endocrinology*, **30**, 544–546.

McCracken, J.A., Baird, D.J. and Goding, J.R. (1971) Factors affecting the secretion of steroids from the transplanted ovary in the sheep. *Recent Progress in Hormone Research*, **27**, 537–582.

McCracken, J.A., Schramm, W., Barcikowski, L. and Wilson, L. (1981) The identification of prostaglandin $F_{2\alpha}$ as a uterine luteolytic hormone and the hormonal control of its synthesis. *Acta Veterinary Scandinavia*, Suppl. 77, 71–88.

McEwen, B.S. (1982) Sexual differentiation of the brain: gonadal hormone action and current concepts of neuronal differentiation of the brain, in *Molecular Approaches to Neurobiology* (ed I.R. Brown), Academic Press, New York.

McEwen, B.S. (1983) Gonadal steroid influences on brain development and sexual differentiation. *International Reviews of Physiology*, **27**, 99–145.

McEwen, B.S. (1987) Steroid hormones and brain development: Some guidelines for understanding actions of pseudohormones and other toxic agents. *Environmental Health Perspectives*, **74**, 177–184.

McEwen, B.S. (1988) Actions of sex hormones on the brain: 'organization' and 'activation' in relation to functional teratoloty in *Progress in Brain Research*, Vol. 73 (eds G.J. Boer, M.G.P. Feenstra, M. Mirmiran, D.F. Swaab and F. Haaren), Elsevier Science Publishers Biomedical Division, New York, pp. 121–134.

McFeely, R.A. (1967) Chromosome abnormalities in early embryos of the pig. *Journal of Reproduction and Fertility*, **13**, 579–581.

McFeely, R.A. and Biggers, J.D. (1965) A rare case of female pseudohermaphroditism in the dog. *Veterinary Record*, **77**, 696–698.

McFeely, R.A. and Rajakoski, E. (1968) Chromosome studies on early embryos of the cow. *Proceedings of the Sixth Congress of Animal Reproduction, Paris*, pp. 905–907.

McFeely, R.A., Hare, W.C.D. and Biggers, J.D. (1967) Chromosome studies in 14 cases of intersex in domestic animals. *Cytogenetics*, **6**, 242–253.

McKeown, T., Marshall, T. and Record, R.G. (1976) Influences on fetal growth. *Journal of Reproduction and Fertility*, **47**, 167–181.

McLachlan, J.A. and Newbold, R.R. (1987) Estrogens and development. *Environmental Health Perspectives*, **75**, 25–27.

McLachlan, J.A., Pratt, R.M. and Markert, C.L. (eds) (1987) Banbury Report 26: *Developmental Toxicology: Mechanisms and Risk*, Cold Spring Harbor Laboratory Press, Col Spring Harbor, NY.

McLaren, A. (1992) Development of primordial germ cells in the mouse. *Andrologia*, **24**, 243–247.

McLaren, A. (1974) Fertilization, cleavage and implantation, in *Reproduction in Farm Animals*, 3rd edn (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 143–165.

McLaren, A. (1980) Oocytes in the testis. *Nature*, **283**, 688–689.

McLaren, A. (1983) Studies of mouse germ cells inside and outside the gonad. *Journal of Experimental Zoology*, **228**, 167–171.

McLaren, A. (1984) Where to draw the line. *Proceedings of the Royal Institution of Great Britain*, **56**, 101–121.

McLaren, A. (1985) The control of implantation, in *In vitro Fertilisation and Donor Insemination* (eds W. Thompson, D.N. Joyce and J.R. Newton), Royal College of Obstetricians and Gynaecologists, London, pp. 13–22.

McLaren, A. (1988) The IVF conceptus. *Annals of the New York Academy of Science*, **541**, 639–645.

McLaren, A. (1988) Somatic and germ-cell sex in mammals. *Philosophical Transactions of the Royal Society of London*, Series 322(1208): 3–9.

McLaren, A. (1990) What makes a man a man?, *Nature*, **346**, 216–217.

McLaren, A., Simpson, E., Tomonari, K., Chandler, P. and Hagg, H. (1984) Male sexual differentiation in mice lacking H-Y antigen. *Nature*, **312**, 552–555.

McLean-Morris, J. and Van Wagenen, G. (1966) Compounds interfering with ovum implantation and development. III. The role of oestrogens. *American Journal of Obstetrics and Gynecology*, **96**, 804–815.

McLean-Morris, J. and Van Wagenen, G. (1973) Interception: The use of postovulatory estrogens to prevent implantation. *American Journal of Obstetrics and Gynecology*, **115**, 101–106.

MacLusky, N.J. and Naftolin, F. (1981) Sexual differentiation of the central nervous system. *Science*, **211**, 1294–1303.

McNatty, K.P., Henderson, K.M. and Sawers, R.S. (1975) Effects of prostaglandin $F_{2\alpha}$ and E_2 on the production of progesterone by human granulosa cells in tissue culture. *Journal of Endocrinology*, **67**, 231–240.

Magre, S. and Jost, A. (1980) The initial phases of testicular organogenesis in the rat. An electron microscopy study. *Archives D Anatomie Microscopique et de Morphologie Experimentale*, **69**, 297–298.

Magre, S. and Jost, A. (1984) Dissociation between testicular organogenesis and endocrine cytodifferentiation of Sertoli cells. *Proceedings of the National Academy of Sciences of the USA*, **81**, 7831–7834.

Magre, S., Angelopoulou, R. and Jost, A. (1981) Action du serum de foetus de veau sur la differenciation *in vitro* ou le maintien des cordons seminiferes du testicule du doetus de rat. *Comptes Rendus Hebdomadaires des Séances de L'Academie des Sciences* (Paris), 292 series, **III**, 85–89.

Maik, H. (1969) Chromosomal sex determination in the intersexual pigs, *Sus scrofa domesticus*. *Genetica Polonica*, 10, 172–173.

Manaugh, L.C. and Novy, M.J. (1976) Effects of indomethacin on corpus luteum function and pregnancy in rhesus monkeys. *Fertility and Sterility*, **27**, 588–598.

Mandarim-de-Lacerda, C.A. (1990) Foot length growth related to crown-rump length, gestational age and weight in human staged fresh fetuses. An index for anatomical and medical use. *Surgical and Radiological Anatomy*, **12**, 103–107.

Mandelbrote, B.M. and Monro, M. (1964) Neurotic illness as a factor in reproduction. *Acta Psychiatrica Scandinavica*, **40**, 419–426.

Mandell, F. and Wolfe, L.C. (1974) Sudden infant death syndrome and subsequent pregnancy. *Pediatrics*, **56**, 774–776.

Mares, S.E., Menge, A.C., Tyler, W.J. and Casida, L.E. (1958) Some sources of variation in conception rate and pregnancy loss in parous Holstein cows. *Journal of Animal Science*, **17**, 1217.

Marston, J.H. and Kelly, W.A. (1969) Contraceptive action of intrauterine devices in the ferret. *Journal of Reproduction and Fertility*, **18**, 419–429.

Marston, J.H., Penn, R. and Sivelle, P.C. (1977) Successful autotransfer of tubal eggs in the rhesus monkey, *Macaca mulatta*. *Journal of Reproduction and Fertility*, **49**, 175–176.

Martal, J., Lacroix, M., Loudes, C., Saunier, M. and Wintenberger-Torres, S. (1979) Trophoblastin, an antiluteolytic protein present in early

pregnancy in sheep. *Journal of Reproduction and Fertility*, **56**, 63–73.

Martal, J., Charlier, M., Camous, S., Fevre, J. and Heyman, Y. (1984) Specificity of embryonic signals maintaining corpus luteum in early pregnancy in ruminants. *Proceedings of the 10th International Congress on Animal Reproduction and AI, Urbana*, **3**, University of Illinois, Urbana-Champaign, Illinois, 509.

Marti, U., Burwen, S.J. and Jones, A.L. (1989) Biological effects of epidermal growth factors, with emphasis on the gastrointestinal tract and liver: An update. *Hepatology*, **9**, 126–138.

Martin, P.A. and Shaver, E.L. (1972) Sperm aging in utero and chromosomal anomalie in rabbit blastocysts. *Developmental Biology*, **28**, 480–486.

Maurer, R.R. and Foote, R.H. (1971) Maternal ageing and embryonic mortality in the rabbit. I. Repeated superovulation, embryo culture and transfer. *Journal of Reproduction and Fertility*, **25**, 329–341.

Meck, J.M. (1984) The genetics of the H-Y antigen system and its role in sex determination. *Perspectives in Biology and Medicine*, **27**, 560–584.

Meire, H.B. (1986) Ultrasound measurement of fetal growth, in *Human Growth*, Vol. l, *Developmental Biology Prenatal Growth* (eds F. Falkner and J.M. Tanner), Plenum Press, New York, pp. 275–290.

Mercola, M. and Stiles, C.D. (1988) Growth factor superfamilies and mammalian embryogenesis. *Development*, **102**, 451–460.

Michael, A.E. and Webley, G.E. (1993) The roles of cyclic AMP and inositol phosphates in the luteolytic action of cloprostenol, a prostaglandin F2α analogue, in the marmoset monkey. *Journal of Reproduction and Fertility*, **97**, 425–431.

Migeon, B., Brown, T.R., Axelman, J. and Migeon, C.J. (198la) Studies of the locus for androgen receptor: Localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse. *Proceedings of the National Academy of Sciences of the USA*, **78**, 6339–6343.

Migeon, C.J., Brown, T.R. and Fichman, K.R. (1981b) Androgen insensitivity syndrome, in *The Intersex Child* (ed. N. Josso), Karger, Basle, 71.

Miller, H.C. and Merritt, T.A. (1979) *Fetal growth in humans*, Year Book Medical Publishers, Chicago.

Miller, J.F., Williamson, E., Glue, J., Gordon, Y.B., Grudzinkskas, J.G. and Sykes, A. (1980) Fetal loss after implantation: A prospective study. *Lancet*, **2**, 554–556.

Mintz, D.H., Chez, R.A. and Hutchinson, D.L. (1972) Subhuman primate pregnancy complicated by streptozotocin-induced diabetes mellitus. *Journal of Clinical Investigation*, **51**, 837–847.

Mittwoch, U. (1983) Heterogametic sex chromosomes and the development of the dominant gonad in vertebrates. *American Naturalist*, **122**, 158–180.

Molteni, R.A., Stys, S.J. and Battaglia, F.C. (1978) Relationship of fetal and placental weight in human beings: Fetal/placental weight ratios at various gestational ages and birth weight dimensions. *Journal of Reproductive Medicine*, **21**, 327–334.

Monroe, S.E., Atkinson, L.E. and Knobil, E. (1970) Patterns of circulating luteinizing hormone and their relation to plasma progesterone levels during the menstrual cycle of the rhesus monkey. *Endocrinology*, **87**, 453–455.

Moon, R.G., Rashad, M.N. and Mi, M.P. (1975) An example of polyploidy in pig blastocysts. *Journal of Reproduction and Fertility*, **45**, 147–149.

Moor, R.M. and Rowson, L.E.A. (1966) The corpus luteum of the sheep: effect of removal of embryos on luteal function. *Journal of Endocrinology*, **34**, 497–502.

Moore, H.D.M., Gems, S. and Hearn, J.P. (1985) Early implantation stages in the marmoset monkey (*Callithrix jacchus*). *American Journal of Anatomy*, **172**, 265–278.

Moore, K.L. (1977) *The Developing Human*, W.B. Saunders, Philadelphia.

Moore, K.L. (1982) The developing human in *Clinically Oriented Embryology*, 3rd edn, W.B. Saunders, Philadelphia, pp. 479.

Moore, R.M. and Trounson, A.O. (1977) Hormonal and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity. *Journal of Reproduction and Fertility*, **49**, 101–109.

Morgan, F.J., Birken, S. and Canfield, R.E. (1973) hCG: a proposal for the amino acid sequence. *Molecular and Cellular Biochemistry*, **2**, 87–97.

Morishima, H.O., Pederson, H. and Finster, M. (1978) The influence of maternal psychological stress on the fetus. *American Journal of Obstetrics and Gynecology*, **131**, 286–290.

Morriss, F.H. (1981) Placental factors conditioning fetal nutrition and growth. *American Journal of Clinical Nutrition*, **34**, 760–768.

Morton, H., Rolfe, B.E., McNeill, L., Clarke, P.,

Clarke, F.M. and Clunie, G.J.A. (1980) Early pregnancy factor: tissues involved in its production in the mouse. *Journal of Reproductive Immunolology*, **2**, 73–82.

Morton, H., Morton, D.J. and Ellendorff, F. (1983) The appearance and characteristics of early pregnancy factor in the pig. *Journal of Reproduction and Fertility*, **68**, 437–446.

Moudgal, N.R. (1984) Corpus luteum of the nonhuman primate. *Advances in Veterinary Science and Comparative Medicine*, **28**, 343–366.

Mougdal, N.R., MacDonald, G.J. and Greep, R.O. (1971) The effect of hCG anitiserum on ovulation and corpus luteum function in the monkey. *Journal of Clinical Endocrinology and Metabolism*, **32**, 579–581.

Mowszowciz, I., Stamatiadis, D., Wright, F., Kuttenn, F. and Mauvais-Harvis, P. (1989) Androgen receptor in sexual differentiation. *Journal of Steroid Biochemistry*, **32**, 157–162.

Mueller-Heubach, E., Meyers, R.E. and Adamsons, K. (1972) Effects of adrenalectomy on pregnancy length in the rhesus monkey. *American Journal of Obstetrics and Gynecology*, **112**, 221–226.

Mulay, S., Browne, C.A., Varma, D.R. and Solomon, S. (1980) Placental hormones, nutrition and fetal development. *Federation Proceedings*, **39**, 261–265.

Muller, U. (1984) Chromosomes and sex differentiation in eutherians. *Journal of Embryology and Experimental Morphology*, **83**, (suppl), 41–49.

Muller, U. and Urban, E. (1981) Reaggregation of rat gonadal cells *in vitro*: Experiments on the function of H-Y antigen. *Cytogenetics and Cell Genetics*, **31**, 104–107.

Muller, U., Aschmoneit, I., Zenzes, M.T. and Wolf, U. (1978a) Binding studies of H-Y antigen in rat tissues: Indication for a gonad-specific receptor. *Human Genetics*, **43**, 151–157.

Muller, U., Zenzes, M.T., Bauknecht, T., Wolf, U., Siebers, J.W. and Engel, W. (1978b) Appearance of hCG-receptor after conversion of newborn ovarian cells into testicular structures by H-Y antigen *in vitro*. *Human Genetics*, **45**, 203–207.

Murray, F.J., Schwetz, B.A., Nitschke, K.D., John, J.A., Norris, J.M. and Gehring, P.J. (1978a) Teratogenicity of acrylonitrile given to rats by gavage or by inhalation. *Food and Cosmetics Toxicology*, **16**, 547–551.

Murray, F.J., John, J.A., Balmer, M.F. and Schwetz, B.A. (1978b) Teratologic evaluation of styrene given to rats and rabbits by inhalation or by gavage. *Toxicology*, **11**, 335–343.

Murray, F.J., Nitschke, K.D, Rampy, L.W. and Schwetz, B.A. (1979b) Embryotoxicity and fetotoxicity of inhaled or ingested vinylidene chloride in rats and rabbits. *Toxicology and Applied Pharmacology*, **49**, 189–192.

Murray, F.J., John, J.A., Rampy, L.W., Kuna, R.A. and Schwetz, B.A. (1979a) Embryotoxicity of inhaled benzene in mice and rabbits. *American Industrial Hygiene Assocication Journal*, **40**, 993–998.

Murti, G.S., Gilbert, D.L. and Borgman, A.R. (1966) Canine intersex states. *Journal of the American Veterinary Medical Association*, **149**, 1183–1185.

Myers, R.E. (1972) The pathology of the rhesus monkey placenta. *Acta Endocrinologica*, **71** (Suppl 155), 221–257.

Myers, R.E. (1975) Maternal psychological stress and fetal asphyxia: A study in the monkey. *American Journal of Obstetrics and Gynecology*, **122**, 47–59.

Nagai, Y. and Ohno, S. (1977) Testis-determining H-Y antigen in XO male of the mole-vole (*Ellobius lutescens*). *Cell*, **10**, 729–732.

Nagle, C.A. and Denari, J.H. (1983) The cebus monkey (*Cebus apella*), in *Reproduction in New World Primates* (ed. J.P. Hearn), M.T.P. Press, Lancaster, pp. 39–69.

Nahmias, A.J. and Visintine, A.M. (1976) Herpes simplex, in *Infectious Diseases of the Fetus and Newborn* (eds J.S. Remington and J.O. Klein), W.B. Saunders, Philadelphia, pp. 156–191.

Nahmias, A.J., Josey, W.E., Naib, Z.M., Freeman, M.G., Fernandez, R.J. and Wheeler, J.H. (1971) Perinatal risk associated with maternal herpes simplex virus infections. *American Journal of Obstetrics and Gynecology*, **110**, 825–837.

Nalbandov, A.V. (1958) Effect of progesterone on ovarian morphology and on embryonal mortality in pregnant rats, pigs and sheep. *Annals of the New York Academy of Sciences*, **71**, 580–585.

Nau, H. (1987) Species differences in pharmacokinetics, drug metabolism and teratogenesis, in *Pharmacokinetics in Teratogenesis*, Vol. 1 (eds H. Nau and W.J. Scott), CRC Press, Boca Raton, Florida, pp. 81–106.

Nawrot, P.S. and Staples, R.E. (1979) Embryofetal toxicity and teratogenicity of benzene and toluene in the mouse. *Teratology*, **19**, 41A.

Naylor, A.F. (1974) Sequential aspects of spontaneous abortion: Maternal age, parity, and

pregnancy compensation artifact. *Social Biology*, **21**, 195–204.

Neill, J.D., Johansson, D.B. and Knobil, E. (1969) Failure of hysterectomy to influence the normal pattern of cyclic progesterone secretion in the rhesus monkey. *Endocrinology*, **84**, 464–465.

Nelson, M.M. and Evans, H.M. (1953) Relation of dietary protein levels to reproduction in the rat. *Journal of Nutrition*, **51**, 71–84.

Nes, N. (1966) Testikulaer feminisering hos storfe Nord. *Veterinary Medicine*, **18**, 19–29.

Neubert, D., Barrach, H.J. and Merker, H.J. (1980) Drug-induced damage to the embryo or fetus, in *Current Topics in Pathology*, Vol. 69 (eds C.L. Berry, E. Grundmann and W.H. Kirsten), Springer-Verlag, Berlin, pp. 241–331.

Neumann, F., Kramer, M. and Junkmann, K. (1964) Experimental studies of 17a-ethinyl-19-nortestosterone acetate in animals. *Medicina Experimentalis*, Suppl. 11, 1–36.

Neumann, F., Grat, K.J. and Elger, E. (1974) Hormone-induced disturbances in sexual differentiation, in *Hormones and Embryonic Development* (ed G. Raspe), Pergamon Press, New York, pp. 71–101.

Neuweiler, V.W. (1951) Die hypervitaminose und ihre Bezichung zur Schwangerschaft. *Beiheft zur Internationalen Zeitschrift fur Vitamin-forschung*, **22**, 392–396.

Neuweiler, W. Von. and Richter, R.H.H. (1964) Beitrag zur frage der entstehung kongenitaler anomalien. Bisherige ergebnisse unserer versuche uber die wirkung verschiedener substanzen auf die embryonale und foetale entwicklung der ratte, in *Medikamentlose Pathogenese Fetaler Missbildungen*, Symposium Listal, 1963. Karger, Basle, pp. 47–79.

New, M.I. (1970) Male pseudohermaphroditism due to 17Q-hydroxylase deficiency. *Journal of Clinical Investigation*, **49**, 1930–1941.

New, M.I., Dupont, B., Pang, S., Pollack, M. and Levine, L.S. (1981) An update of congenital adrenal hyperplasia. *Recent Progress in Hormone Research*, **37**, 105–112.

Newbold, R.R. and McLachlan, J.A. (1982) Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. *Cancer Research*, **42**, 2003–2011.

Nicolson, N.A. (1981) Weaning and the development of independence in Olive Baboons. *PhD Thesis*, Harvard University.

Nishihara, G. (1958) Influence of female sex hormones in experimental teratogenesis. *Proceedings of the Society for Experimental Biology and Medicine*, **97**, 809–812.

Nishikawa, Y. and Hafez, E.S.E. (1962) The reproduction of horses, in *Reproduction in Farm Animals* (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 266–276.

Nishimura, H. and Nakai, K. (1958) Developmental anomalies in offspring of pregnant mice treated with nicotine. *Science*, **127**, 877–878.

Nobunaga, T., Satoh, H. and Suzuki, T. (1979) Effects of sodium selenite on methylmercury embryotoxicity and teratogenicity in mice. *Toxicology and Applied Pharmacology*, **47**, 79–88.

Noden, D.M. and deLahunta, A. (1985) *The Embryology of Domestic Species*, Williams & Wilkins, Baltimore.

Nordeen, E.J. and Yahr, P. (1982) Hemispheric asymmetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. *Science*, **218**, 391–394.

Noyes, R.W. and Dickman, Z. (1960) Relationship of ovular age to endometrial development. *Journal of Reproduction and Fertility*, **1**, 186–212.

Ohno, S. (1979) *Major Sex-Determining Genes*, Springer-Verlag, New York.

Ohno, S., Nagai, Y. and Ciccarese, S. (1978) Testicular cells lysostripped of H-Y antigen organize ovarian follicle-like aggregates. *Cytogenetics and Cell Genetics*, **20**, 351–364.

O'Neill, C. (1985) Examination of the causes of early pregnancy-associated thrombocytopenia in mice. *Journal of Reproduction and Fertility*, **73**, 567–577.

O'Neill, C. (1987) Embryo-derived platelet activating factor: A preimplantation embryo mediator of maternal recognition of pregnancy. *Domestic Animal Endocrinology*, **4**, 69–85.

O'Neill, C., Gidley-Baird, A.A., Pike, I.L., Porter, R.N., Sinosich, M.J. and Saunders, D.M. (1985) Maternal blood platelet physiology and luteal-phase endocrinology as a means of monitoring pre- and post-implantation embryo viability following *in vitro* fertilisation. *Journal of in vitro Fertilisation and Embryo Transfer*, **2**, 87–93.

Oppe, T.E., Hsia, Dy-y. and Gellis, S. (1957) Pregnancy in the diabetic mother with nephritis. *Lancet*, **1**, 353–354.

O'Rahilly, R. (1973) Developmental stages in human embryos. Part A: Embryos of the first three weeks (stages 1–9). *Contributions to Embryology*, Carnegie Institute, Washington, Publication 531.

O'Rahilly, R. (1977) The development of the vagina in the human, in *Morphogenesis and Malformation of the Genital System* (eds R.J. Blandau and D. Bergsma), Alan R. Liss, New York, pp. 123–136.

O'Rahilly, R. (1983) The timing and sequence of events in the development of the human reproductive system during the embryonic period proper. *Anatomy and Embryology*, **166**, 247–261.

O'Rahilly, R. and Müller, F. (1986) Human growth during the embryonic period proper, in *Human Growth*, Vol. 1, Developmental Biology Prenatal Growth (eds F. Falkner and J.M. Tanner), Plenum Press, New York, pp. 245–253.

O'Rahilly, R. and Müller F. (1987) *Developmental Stages in Human Embryos. Contributions to Embryology*, Carnegie Institution of Washington, Publication 637.

Ornoy, A. (1973) Transplacental effects of estrogen on osteogenesis in rat foetuses. *Pathology*, **5**, 183–188.

Orozco, C., Perkins, T. and Clarke, F.M. (1986) Platelet-activating factor induces the expression of early factor activity in female mice. *Journal of Reproduction and Fertility*, **78**, 549–555.

Ortiz, M.E. and Croxatto, H.B. (1979) Observations on the transport, aging and development of ova in the human genital tract, in *Recent Advances in Reproduction and Regulation of Fertility* (ed. G.P. Talwar), Elsevier North Holland, Amsterdam, pp. 307–317.

Osebold, J.W. (1977) Infectious diseases influencing reproduction, in *Reproduction in Domestic Animals*, (eds H.H. Cole and P.T. Cupps), Academic Press, New York, pp. 605–629.

Ottobre, J.S., Ottobre, A.C. and Stouffer, R.L. (1984) Changes in available gonadotropin receptors in the corpus luteum of the rhesus monkey during simulated early pregnancy. *Endocrinology*, **115**, 198–204.

Palmer, A.K. (1978) Rabbits (developmental abnormalities), in *Pathology of Laboratory Animals*, (eds K. Benirschke, F.M. Garner and T.C. Jones), Vol. 2, Springer-Verlag, New York, pp. 1848–1860.

Page, E.W., Villee, C.A. and Villee, D.B. (1981) Human Reproduction, in *Essentials of Reproductive and Perinatal Medicine*, 3rd edn, W.B. Saunders, Philadelphia.

Pang, S., Levine, L.S., Stoner, E., Opitz, J.M., Pollack, M.S., Dupont, B. and New, M.I. (1983) Non- salt-losing congenital adrenal hyperplasia due to 3 R-hydroxysteroid dehydrogenase deficiency with normal glomerulosa function. *Journal of Clinical Endocrinology and Metabolism*, **56**, 808–818.

Parkinson, T.J., Lamming, G.E., Flint, A.P.F. and Jenner, L. (1992) Administration of recombinant bovine interferon-α_1 at the time of maternal recognition of pregnancy inhibits prostaglandin $F_{2\alpha}$ secretion and causes luteal maintenance in cyclic ewes. *Journal of Reproduction and Fertility*, **94**, 489–500.

Pate, J.L. and Condon, W.A. (1984) Effects of prostaglandin $F_{2\alpha}$ on agonist-induced progesterone production in cultured bovine luteal cells. *Biology of Reproduction*, **31**, 427–435.

Pathak, S. and Stock, A.D. (1976) Giemsa-binding and the identification of the Y/autosome translocation in the African marsh mongoose, *Atilax palindinosus* (Carnivora Viverridae). *Cytogenetics and Cell Genetics*, **16**, 487–494.

Patten, B.M. (1953) *Embryology of the Pig*, Blakeston, Philadelphia.

Patten, B.M. (1968) *Human Embryology*, 3rd edn, McGraw-Hill, New York.

Pelliniemi, L.J. and Niemi, M. (1969) Fine structure of the human foetal testis. I. The interstitial tissue. *Zeitschrift fur Zellforschung Mikroskopische Anatomie*, **99**, 507–522.

Perry, J.S. (1945) The reproduction of the wild brown rat (*Rattus norvegicus Erxleben*). *Proceedings of the Zoological Society of London*, **115**, 19–46.

Perry, J.S. (1954) Fecundity and embryonic mortality in pigs. *Journal of Embryology and Experimental Morphology*, **2**, 308–322.

Perry, J.S. (1960) The incidence of embryonic mortality as a characteristic of the individual sow. *Journal of Reproduction and Fertility*, **1**, 71–83.

Perry, J.S., Heap, R.B. and Amoroso, E.C. (1973) Steroid hormone production by pig blastocysts. *Nature*, **245**, 45–47.

Persaud, T.V.N., Chudley, A.E. and Skalko, R.G. (1985) *Basic Concepts in Teratology*, Alan R. Liss, New York, p. 190.

Peterson, D.L. and Edgren, R.A. (1965) The effect of various steroids on mating behaviour, fertility and fecundity of rats. *International Journal of Fertility*, **10**, 327–332.

Peterson, R.E., Imperato-McGinley, J., Gautier, T. and Sturla, E. (1977) Male pseudohermaphroditism due to steroid 5 a-reductase deficiency. *American Journal of Medicine*, **62**, 170–191.

Phillips, J.M., Brief, B.J., Sutton, T.S. and Mills,

J.W. (1939) Hermaphroditism. *Journal of American Veterinary Medical Association*, **95**, 663–674.

Phoenix, C.H., Goy, R.W., Gerall, A.A. and Young, W.C. (1959) Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, **65**, 369–388.

Picon, L. (1967) Effect of insulin on growth and biochemical composition of the rat fetus. *Endocrinology*, **81**, 1419–1421.

Picon, R. (1969) Action du testicule foetal sur le développement *in vitro* des canaux de Muller chez le rat. *Archeves d'Anatomie Microscopique*, **58**, 1–19.

Picard, J.Y., Tran, D. and Josso, N. (1978) Biosynthesis of labelled anti-Müllerian hormone by fetal testes: Evidence for the glycoprotein nature of the hormone and for its disulfide-bonded structure. *Molecular and Cellular Endocrinology*, **12**, 17–30.

Pierce, J.G., Liao, T.H., Howard, S.M., Shome, B. and Cornell, J.S. (1971) Studies on the structure of thyrotrophin: its relationship to luteinising hormone. *Recent Progress in Hormone Research*, **27**, 165–206.

Piotrowski, J. (1969) Experimental studies on the effect of some steroid hormones on the development of the rabbit foetus. *Przeglad Lekarski*, **25**, 324–332.

Piotrowski, J. (1971) Badania doswiadczalne nad wplywem hormonow sterydowych na rozwoj embrionalny. *Folia Medica Cracoviensia*, **13**, 251–280.

Plante, C., Bousquet, D., Guay, P. and Goff, A.K. (1987) Luteotrophic activity of bovine embryos. *Theriogenology*, **28**, 801–813.

Plante, C., Hansen, P.J. and Thatcher, W.W. (1988) Prolongation of luteal lifespan in cows by intrauterine infusion of recombinant alpha-interferon. *Endocrinology*, **122**, 2342–2344.

Polani, P.E. (1974) Chromosomal and other genetic influences on birth weight variation, in *Size at Birth*, Vol. 27 (eds K. Elliot and J. Knight), Ciba Foundation Symposia, Elsevier-Excerpta Medica, Amsterdam, pp. 127.

Polani, P.E. (1981a) Anomalies of sex-differentiating mechanisms, in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 549–590.

Polani, P.E. (1981b) Abnormal sex development in man, in *Mechanisms of Sex Differentiation in Animals and Man* (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 465–548.

Polge, C. (1985) Embryo manipulation and genetic engineering. *Symposium of the Zoological Society of London*, **54**, 123–135.

Policastro, P.F., Daniels-McQeen, S., Carle, G. and Boime, I. (1986) A map of the hCGβ/LHβ gene cluster. *Journal of Biological Chemistry*, **261**, 5907–5916.

Polishuk, W.Z., Siew, F.P., Gordon, R. and Lebenshart, P. (1977) Vascular changes in traumatic amenorrhea and hypomenorrhea. *International Journal of Fertility*, **22(3)**, 189–192.

Pomerantz, S.M., Roy, M.M. and Goy, R.W. (1988) Social and hormonal influences of behavior of adult male, female and pseudohermaphroditic rhesus monkeys. *Hormones and Behavior*, **22**, 219–230.

Pontzer, C.H., Torres, B.A., Vallet, J.L., Bazer, F.W. and Johnson, H.M. (1988) Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. *Biochemical Biophysical Research Communications*, **152**, 801–807.

Pope, C.E., Pope, V.A. and Beck, L.R. (1982) Development of baboon preimplantation in embryos to postimplantation stages *in vitro*. *Biology of Reproduction*, **27**, 915–923.

Pope, C.E., Pope, V.A. and Beck, L.R. (1984) Live birth following cryopreservation and transfer of a baboon embryo. *American Fertility Society*, **42**, 143–145.

Prahalada, S. and Hendrickx, A.G. (1983) Embryotoxicity of Norlestrin, a combined synthetic contraceptive, in rhesus macaques (*Macaca mulatta*). *Teratology*, **27**, 215–222.

Prahalada, S., Carroad, E. and Hendrickx, A.G. (1985b) Embryotoxicity and maternal serum concentrations of medroxyprogesterone acetate (MPA) in baboons (*Papio cynocephalus*). *Contraception*, **32**, 497–515.

Prahalada, S., Carroad, E., Cukierski, M. and Hendrickx, A.G. (1985a) Embryotoxicity of a single dose of medroxyprogesterone acetate (MPA) and maternal serum concentrations in cynomolgus monkey (*Macaca fascicularis*). *Teratology*, **32**, 421–432.

Pratt, B.R., Butcher, R.L. and Inskeep, E.K. (1977) Antiluteolytic effect of the conceptus and PGE_2 in ewes. *Journal of Animal Science*, **46**, 784–791.

Preston, P.G. (1942) Six years maternity amongst the Wakikuyu at the native hospital, Fort Hall. *East African Medical Journal*, **19**, 247–257.

Price, C.J., Kimmel, C.A., Tyl, R.W. and Marr, M. (1985) The developmental toxicity of ethylene glycol in rats and mice. *Toxicology and Applied Pharmacology*, **81**, 113–127.

Price, D., Zaaijer, J.J.P., Ortiz, E. (1968) Prenatal development of the oviduct *in vivo* and *in vitro*, in *The Mammalian Oviduct* (eds E.S.E. Hafez and R.J. Blandau), University of Chicago Press, Chicago, pp. 29–46.

Price, J.M., Donahoe, P.K., Ito, Y. and Hendren, W.H. III (1977) Programmed cell death in the mullerian duct induced by mullerian inhibiting substance. *American Journal of Anatomy*, **149**, 353–376.

Pullen, C.M. (1970) True bilateral hermaphroditism in a beagle: A case report. *American Journal of Veterinary Research*, **31**, 1113–1117.

Quadagno, D.M., Briscoe, R. and Quadagno, J.S. (1977) Effect of perinatal gonadal hormones on selected nonsexual behavior patterns: A critical assessment of the nonhuman and human literature. *Psychological Bulletin*, **84**, 62–80.

Quinlivan, T.D., Martin, C.A., Taylor, W.B. and Cairney, I.M. (1966) Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. I. Pre- and perinatal mortality in those ewes that conceived to one service. *Journal of Reproduction and Fertility*, **11**, 379–390.

Ragsdale, A.C., Brody, S., Thompson, H.J. and Worstell, D.M. (1948) Environmental physiology with special reference to domestic animals. II. Influence of temperature, 50° to 105°F, on milk production and feed consumption in dairy cattle. *Missouri Agricultural Experimental Station*, Research Bulletin, 425.

Ragule, N. (1974) Embryotropic action of styrene. *Gigiena i Sanitariya*, **11**, 85–86.

Rainbow, T.C., Parsons, B. and McEwen, B.S. (1982) Sex differences in rat brain oestrogen and progestin receptors. *Nature*, **300**, 648–649.

Raisman, G. and Field, P.M. (1973) Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. *Brain Research*, **54**, 1–29.

Randall, C.L., Taylor, W.J. and Walker, D.W. (1977) Ethanol-induced malformations in mice. *Alcoholism: Clinical and Experimental Research*, **1**, 219–224.

Rattray, P.V. (1977) Nutrition and reproductive efficiency, in *Reproduction in Domestic Animals* (eds H.H. Cole and P.T. Cupps), Academic Press, New York, pp. 553–575.

Raynaud, A. (1942) Inhibition de l'allongement et de la soudure des paupières des embryons de souris. (Inhibition of eyelid development in mouse embryos). *Comptes Rendus des Séances de la Société de Biologie*, **136**, 337–338.

Raynaud, A. (1958) Inhibition of the development of the gubernaculum of the male mouse foetus under the influence of an oestrogenic hormone. *Compte Rendu Hebdomadaire de l'Academie des Sciences, Paris*, **246**, 176–179.

Reece, E.A., Gabrielli, S., Degennaro, N. and Hobbins, J.C. (1989) Dating through pregnancy: A measure of growing up. *Obstetrics and Gynecological Survey*, **44**, 544–555.

Reindollar, R.H., Tho, S.P.T. and McDonough, P.G. (1987) Abnormalities of sexual differentiation: Evaluation and management. *Clinical Obstetrics and Gynecology*, **30**, 697–713.

Reinisch, J. (1977) Prenatal exposure of human fetuses to synthetic progestin and oestrogen affects personality. *Nature*, **266**, 561–562.

Remington, J.S. and Klein, J.O. (eds) (1983) *Infectious Diseases of the Fetus and Newborn Infant*, W.B. Saunders, Philadelphia, pp. 1147.

Resnik, R. (1978) Maternal diseases associated with abnormal fetal growth. *Journal of Reproductive Medicine*, **21**, 315–318.

Richardson, M.C. (1986) Hormonal control of ovarian luteal cells, in *Oxford Reviews of Reproductive Biology* (ed. J.R. Clarke), Clarendon Press, Oxford, pp. 321–378.

Richardson, M.C. and Masson, G.M. (1980) Progesterone production by dispersed cells from human corpus luteum: stimulation by gonadotrophins and prostaglandin $F_{2\alpha}$: lack of response to adrenaline and isoprenaline. *Journal of Endocrinology*, **87**, 247–254.

Richardson, M.C. and Masson, G.M. (1981) Stimulation of human chorionic gonadotrophin of oestradiol production by dispersed cells from human corpus luteum: comparison with progesterone production; utilization of exogenous testosterone. *Journal of Endocrinology*, **91**, 197–203.

Richardson, M.C. and Masson, G.M. (1985) Lack of direct inhibitory action of oxytocin on progesterone production by dispersed cells from human corpus luteum. *Journal of Endocrinology*, **104**, 149–151.

Rider, V. and Heap, R.B. (1986) Heterologous antiprogesterone monoclonal antibody arrests early embryonic development and implantation in the ferret (*Mustela putorius*). *Journal of Reproduction and Fertility*, **76**, 459–470.

Rieck, G.W. (1973) Numerical aberrations of gonosomes and reproductive failure in cattle, in *Les Accidents Chromosomiques de la Reproduction* (eds A. Boue and C. Thibault), INSERM, Paris, pp. 165-187.

Rimoin, D.L and Graham, J.M. (1989) Syndromes associated with growth deficieny. *Acta Paediatrica Scandiavica*, Suppl. 349, 3–10.

Riopelle, A.J. (1985) Prenatal protein deprivation, in *Nonhuman Primate Models for Human Growth and Development* (ed. E.S. Watts), Alan R. Liss, New York, pp. 171-185.

Rizzino, A. (1987) Defining the roles of growth factors during early mammalian development, in *The Mammalian Preimplantation Embryo* (ed. B.D. Bavister), Plenum Press, New York, pp. 151-174.

Rizzino, A. (1988) Transforming growth factor-beta: Multiple effects on cell differentiation and extracellular matrices. *Developmental Biology*, **130**, 411–422.

Rizzo, A.M. and Furst, A. (1972) Mercury teratogenesis in the rat. *Proceedings of the Western Pharmacological Society*, **15**, 52–54.

Robboy, S.J., Scully, R.E., Welch, W.R. and Herbst, A.L. (1977) Intrauterine diethylstilbestrol exposure and its consequences. *Archives of Pathology and Laboratory Medicine*, **101**, 1–5.

Roberts, D.F. (1981) Genetics of growth. *British Medical Bulletin*, **37(3)**, 239–246.

Robins, S.A. (1972) Uterotubography, in *Golden's Diagnostic Roentgenology* (ed. L.L. Robbins), Williams & Wilkins, Baltimore, pp. 9.1–9.7.

Robinson, T.J. (1957) Pregnancy, in *Progress in the Physiology of Farm Animals*, Vol. 3 (ed. J. Hammond), Butterworths, London, pp. 793–904.

Rock, J.A. and Jones Jr., H.W. (1977) The clinical management of the double uterus. *Fertility and Sterility*, **28**, 798–806.

Rock, J.A. and Zacur, H.A. (1983) The clinical management of repeated early pregnancy wastage. *Fertility and Sterility*, **39**, 123–140.

Rocklin, R.E., Kitzmiller, J.L., Carpenter, C.B., Garovoy, M.R. and David, J.R. (1976) Maternal–fetal relation: Absence of an immunologic blocking factor from the serum of women with chronic abortions. *New England Journal of Medicine*, **295**, 1209–1213.

Rolfe, B.E. (1982) Detection of fetal wastage. *Fertility and Sterility*, **37**, 655–660.

Rosenstein, L., Bruce, A., Rogers, N. and Lawrence, S. (1977) Neurotoxicity of kepone in perinatal rats following in utero exposure. *Toxicology and Applied Pharmacology*, **41**, 142–143.

Rosenthal, S.M., Grumbah, M.M. and Kaplan, S.L. (1983) Gonadotropin-independent familial sexual precocity with premature Leydig and germinal cell maturation (familial testotoxicosis): Effects of a potent luteinizing hormone-releasing factor agonist and medroxyprogesterone acetate therapy in four cases. *Journal of Clinical Endocrinology and Metabolism*, **57(3)**, 571–579.

Ross, G.T. (1979) Human chorionic gonadotrophin and maternal recognition of pregnancy, in *Maternal Recognition of Pregnancy*, Ciba Foundation Symposium No 64, Excerpta Medica, Amsterdam, pp. 191–298.

Rothchild, I. (1981) The regulation of the mammalian corpus luteum. *Recent Progress in Hormone Research*, **37**, 183–298.

Rotmensch, J., Frey, K. and Herbst, A.L. (1988) Effects on female offspring and mothers after exposure to diethylstilbestrol in *Toxicity of Hormones in Perinatal Life* (eds T. Mori and H. Nagasawa), CRC Press, Boca Raton, Florida, pp. 143–159.

Rowson, L.E.A., Lawson, R.A.S. and Moore, R.M. (1971) Production of twins in cattle by egg transfer. *Journal of Reproduction and Fertility*, **25**, 261–268.

Roy, S.K. and Kar, A.B. (1967) Foetal effects of norethynodrel in rats. *Indian Journal of Experimental Biology*, **5**, 14–16.

Ruch, T.C. (1959) *Diseases of Laboratory Primates*, W.B. Saunders, Philadelphia.

Rushton, D.I. (1985) The nature and causes of spontaneous abortions with normal karyotypes, in *Issues and Reviews in Teratology*, Vol. 3 (ed. H. Kalter), Plenum Press, New York, pp. 21–63.

Rushton, D. (1987) Pathology of abortion: Correlation of chromosomal abnormalities and morphological anomalies in *Haines & Taylor Obstetrical and Gynaecological Pathology* (ed. H. Fox), Churchill Livingstone, New York, pp. 1131–1134.

Russell, M.H., Wachtel, S.S., Davis, B.W., Cahill, L.T., Groos, E., Niblack, G.D. and Burr, I.M. (1982) Ovarian development in 46 X,Y gonadal dysgenesis. *Human Genetics*, **60**, 196–199.

vom Saal, F.S. (1989) Sexual differentiation in litter-bearing mammals: Influence of sex adjacent fetuses in utero. *Journal of Animal Science*, **67**, 1824–1840.

Sadler, T.W. (1985) *Langman's Medical Embryology*, 5th edn, Williams & Wilkins, Baltimore, pp. 408.

Saenger, P. (1984) Abnormal sex differentiation. *Journal of Pediatrics*, **104**, 1–17.

Saenger, P., Levin, L.S., Pang, S. and New, M.I. (1980) Sexual ambiguity at birth. In *Diagnosis in Andrology, Clinics in Andrology* (eds. J. Bailey and E.S.E. Hafez), Martinus Nijhoff, The Hague, The Netherlands, p. 31.

Saenger, P., Levine, L.S., Wachtel, S.S., Korth-Schutz, S., Doberne, Y., Koo, G.C., Lavengood, R.W. German, J. and New MI. (1976) Presence of H-Y antigen and testis in 46, XX true hermaphroditism: Evidence for Y-chromosomal function. *Journal of Clinical Endocrinology and Metabolism*, **43**, 1234–1239.

Sampaio, F.J., Aragao, A.H. (1990) Study of the fetal kidney length growth during the second and third trimesters of gestation. *European Urology*, **17**, 62–65.

Sargent, E.L., Baughman, W.L., Novy, M.J. and Stouffer, R.L. (1988) Intraluteal infusion of a prostaglandin syntheisi inhibitor, sodium meclofenamate causes premature luteolysis in rhesus monkeys. *Endocrinology*, **123**, 2261–2269.

Sarram, M., Feiz, J., Foruzandeh, M. and Gazanfarpour, P. (1974) Intrauterine fetal infection with *Brucella melitensis* as a possible cause of second-trimester abortion. *American Journal of Obstetrics and Gynecology*, **119**, 657–660.

Saunders, F.J. and Elton, R.L. (1967) Effects of ethynodiol diacetate and mestranol in rats and rabbits, on conception, on the outcome of pregnancy and on the offspring. *Toxicology and Applied Pharmacology*, 11, 229–244.

Saxena, B.B., Hasan, S.H., Haour, I. and Scmidt-Gollwitzer, M. (1974) Radioreceptor assay of hCG: detection of early pregnancy. *Science*, **184**, 793–795.

Scammon, R.E. and Calkins, L.A. (1929) *The Development and Growth of the External Dimensions of the Human Body in the Fetal Period*, University of Minnesota Press, Minneapolis.

Schachter, J. (1967) Isolation of Bedsoniae from human arthritis and abortion tissues. *American Journal of Ophthalmology*, **63**, 1082–1086.

Schachter, B. and Toran-Allerand, C.D. (1982) Intraneural alpha-fetoprotein and albumin are not synthesized locally in developing brain. *Developmental Brain Research*, **5**, 93–98.

Schardein, J.L. (1976) *Drugs as Teratogens*, CRC Press, Florida, pp. 291.

Schardein, J.L. (1980) Congenital abnormalities and hormones during pregnancy. A clinical review. *Teratology*, **22**, 251–270.

Schardein, J.L. (1985) *Chemically Induced Birth Defects*, Marcel Dekker, New York.

Schardein, J.L. and Keller, K.A. (1989) Potential human developmental toxicants and the role of animal testing in their identification and characterization. *CRC Critical Reviews in Toxicology*, **19**, 251–339.

Schempp, W., Wiberg, U. and Fredga, K. (1985) Correlation between sexual phenotype and X-chromosome inactivation pattern in the XXY wood lemming. *Cytogenetics and Cell Genetics*, **39**, 30–34.

Schenker, J.G. and Margalioth, E.J. (1982) Intrauterine adhesions: An updated appraisal. *Fertility and Sterility*, **37(5)**, 593–610.

Schlafke, S. and Enders, A.C. (1975) Cellular basis of interaction between trophoblast and uterus at implantation. *Biology of Reproduction*, **12**, 41–65.

Schneider, G., Genel, M., Bongiovanni, A.M., Goldman, A.S. and Rosenfeld, R.L. (1975) Persistent testicular 45-isomerase-3R-hydroxysteroid dehydrogenase (45-3R-HSD) deficiency in the 45-3R-HSD form of congenital adrenal hyperplasia. *Journal of Clinical Investigation*, **55**, 681–690.

Schoonmaker, J.N., Bergman, K.S., Steiner, R.A. and Karsch, F.J. (1982) Estradiol-induced luteal regression in the rhesus monkey: evidence for an extraovarian site of action. *Endocrinology*, **110**, 1708–1715.

Schultz, M.G. (1962) Male pseudohermaphroditism diagnosed with aid of sex chromatin technique. *Journal of American Veterinary Medical Association*, **140**, 241–244.

Schwetz, B.A., Keeler, P.A. and Gehring, P.J. (1974a) Effect of purified and commercial grade tetrachlorophenol on rat embryonal and fetal development. *Toxicology and Applied Pharmacology*, **28**, 146–150.

Schwetz, B.A., Keeler, P.A. and Gehring, P.J. (1974b) The effect of purified and commercial grade pentachlorophenol on rat embryonal and fetal development. *Toxicology and Applied Pharmacology*, **28**, 151–161.

Schwetz, B.A., Leong, B.K. and Gehring, P.J. (1974c) Embryo- and fetotoxicity of inhaled carbon tetrachloride 1,1-dichloroethane and methyl ethyl ketone in rats. *Toxicology and Applied Pharmacology*, **28**, 452–464.

Schwetz, B.A., Smith, F.A., Leong, B.K.J. and Staples, R.E. (1979) Teratogenic potential of

inhaled carbon monoxide in mice and rabbits. *Teratology*, **19**, 385–392.

Scofield, A.M. (1975) Embryonic mortality in the pig, in *Veterinary Annual*, Vol. 15 (eds C.S.G. Grunsell and F.W.G. Hill), Wright, Bristol, pp. 91–94.

Scott, J.R., Rote, N.S. and Branch, D.W. (1987) Immunologic aspects of recurrent abortion and fetal death. *Obstetrics and Gynecology*, **70**, 645–656.

Scott, W.J. (1970) Effects of intrauterine administration of acetazolamide in rats. *Teratology*, **3**, 261–268.

Scott, W.J. (1978) Sheep (developmental abnormalities), in *Pathology of Laboratory Animals*, Vol. 2 (eds K. Benirschke, F.M. Garner and T.C. Jones), Springer-Verlag, New York, pp. 1906–1911.

Seegmiller, R.E., Nelson, G.W. and Johnson, C.K. (1983) Evaluation of the teratogenic potential of delalutin (17-hydroxyprogesterone caproate) in mice. *Teratology*, **28**, 201–208.

Selby, L.A. (1978) Pigs (developmental abnormalities), in *Pathology of Laboratory Animals*, Vol. 2 (eds K. Benirschke, F.M. Garner and T.C. Jones), Springer-Verlag, New York, pp. 1897–1905.

Selden, J.R. and Wachtel, S.S. (1977) H-Y antigen in the dog. *Transplantation*, **24**, 298–299.

Selden, J.R., Wachtel, S.S., Koo, G.C., Haskins, M.E. and Patterson, D.F. (1978) Genetic basis of XX male syndrome and XX true hermaphroditism: Evidence in the dog. *Science*, **201**, 644–646.

Self, H.L., Grummer, R.H. and Casida, L.E. (1955) The effects of various sequences of full and limited feeding on the reproductive phenomena in Chester White and Poland China gilts. *Journal of Animal Science*, **14**, 573–592.

Selvin, S. and Garfinkel, J. (1976) Paternal age, maternal age and birth order and the risk of a fetal loss. *Human Biology*, **48**, 223–230.

Seshagiri, P.B. and Hearn, J.P. (1993) *in vitro* development of *in vivo* produced rhesus monkey morulae and blastocysts of hatched, attached, and post-attached blastocyst stages: morphology and early secretion of chorionic gonadotrophin. *Human Reproduction*, **8**, 279–287.

Seshagiri, P.B., Bridson, W.E., Dierschke, D.J., Eisele, S.G. and Hearn, J.P. (1993) Non-surgical uterine flushing for the recovery of pre-implantation embryos in rhesus monkeys: lack of seasonal infertility. *Americal Journal of Primatology*, **29**, 81–91.

Sever, J.L. (1980) Infectious causes of human reproductive loss, in *Human Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 169–175.

Shah, M.K. (1956) Reciprocal egg transplantation to study embryo–uterine relationship in heat-induced failure of pregnancy in rabbits. *Nature*, **177**, 1134–1135.

Shapiro, B.H. and Goldman, A.S. (1981) New thoughts on sexual differentiation of the brain, in *Genetic Mechanisms of Sexual Development* (eds H. Vallet and I.M. Porter), Academic Press, New York, pp. 221–246.

Shaver, E.L. (1967) Two cases of intersex in rabbits. *Anatomical Record*, **159**, 127–130.

Sheldrick, E.L. and Flint, A.P.F. (1985) Endocrine control of uterine oxytocin receptors in the ewe. *Journal of Endocrinology*, **106**, 249–258.

Shenefelt, R.E. (1978) Golden hamster (developmental abnormalities), in *Pathology of Laboratory Animals*, Vol. 2 (eds K. Benirschke, F.M. Garner and T.C. Jones), Springer-Verlag, New York, pp. 1866–1869.

Sheppard, E.M. (1943) The reproductive system of a pregnant hermaphrodite rabbit (*Oryctolagus cuniculus*). *Journal of Anatomy*, **77**, 288–293.

Shiota, K., Uwabe, C. and Nishimura, H. (1987) High prevalence of defective human embryos at the early postimplantation period. *Teratology*, **35**, 309–316.

Short, R.D., Minor, J.L., House, W.B., Marcus, W. and Lee, C.C. (1976) Continuous inhalation of 1,1-dichloroethylene (DCE) by rats and mice during gestation. *Pharmacologist*, **18**, 245.

Short, R.V. (1979) When a conception fails to become a pregnancy, in *Maternal Recognition of Pregnancy*, Ciba Foundation Symposium 64, Excerpta Medica, Amsterdam, pp. 377–394.

Short, R.V. (1982) Sex determination and differentiation, in *Reproduction in Mammals 2: Embryonic and Fetal Development*, 2nd edn (eds C.R. Austin and R.V. Short), Cambridge University Press, Cambridge, pp. 70–112.

Siiteri, P.K. and Wilson, J.D. (1974) Testosterone formation and metabolism during male sexual differentiation in the human embryo. *Journal of Clinical Endocrinology*, **38**, 113–125.

Silvia, W.J., Fitz, T.A., Mayan, M.H. and Niswender, G.D. (1984) Cellular and molecular mechanisms involved in luteolysis and maternal recognition of pregnancy in the ewe. *Animal Reproduction Science* **7**, 57–74.

Simpson, E. (1982) Sex reversal and sex determination. *Nature*, **300**, 403.

Simpson, E., McLaren, A., Chandler, P. and Tomonari, K. (1984) Expression of H-Y antigen by female mice carrying Sxr. *Transplantation*, **37**, l7.

Simpson, E., Chandler, P., Hunt, R., Hogg, H., Tomonari, K. and McLaren, A. (1986) H-Y status of X/X Sxr male mice: *in vivo* tests. *Immunology*, **57**, 345–349.

Simpson, J.L. (1976) *Disorders of Sexual Differentiation: Etiology and Clinical Delineation*, Academic Press, New York, pp. 158, 183, 226, 260.

Simpson, J.L. (1990) Incidence and timing of pregnancy losses: Relevance to evolutionary safety of early prenatal diagnosis. *American Journal of Medical Genetics*, **35**, 165–173.

Simpson, J.L., Blagowidow, N. and Martin, A.O. (198l) XY gonadal dysgenesis: Genetic heterogeneity based upon clinical observations, H-Y antigen status and segregation analysis. *Human Genetics*, **58**, 91–97.

Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A-M, Lovell-Badge, R. and Goodfellow, P.N. (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, **346**, 240–244.

Singh, A.R., Lawrence, W.H. and Autian, J. (1972a) Embryonic–fetal toxicity and teratogenic effects of a group of methacrylate esters in rats. *Journal of Dental Research*, **51**, 1632–1638.

Singh, A.R., Lawrence, W.H. and Autian, J. (1972b) Teratogenicity of phthalate esters in rats. *Journal of Pharmacological Science*, **61**, 51–55.

Singh, L. and Jones, K.W. (1982) Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. *Cell*, **28**, 205–216.

Singh, L., Phillips, C. and Jones, K.W. (1984) The conserved nucleotide sequences of Bkm, which define Sxr in the mouse, are transcribed. *Cell*, **36**, 111–120.

Sleight, S.D. and Atallah, O.A. (1968) Reproduction in the guinea pig as affected by chronic administration of potassium nitrite and potassium nitrate. *Toxicology and Applied Pharmacology*, **12**, 179–185.

Sloan, W.R. and Walsh, P.C. (1976) Familial persistent Müllerian duct syndrome. *Journal of Urology*, **115**, 459–461.

Small, M.F. (1982) Reproductive failure in macaques. *American Journal of Primatology*, **2**, 137–147.

Smart, Y.C., Roberts, T.K., Clancy, R.L. and Cripps, A.W. (1981) Early pregnancy factor: Its role in mammalian reproduction – research review. *Fertility and Sterility*, **35**, 397–402.

Smidt, D. (1962) Sexualpotenz urd Fruchbarkeitsvererbung beim Schwein. Munich: BLV Verlagsgesellschaft.

Smith, C.A., Moore, H.D.M. and Hearn, J.P. (1987) The ultrastructure of early implantation in the marmsoet monkey (*Callithrix jacchus*). *Anatomy and Embryology*, **175**, 399–410.

Snellings, W.M., Pringle, J.L., Dorko, J.D. and Kintigh, W.J. (1979) Teratology and reproduction studies with rats exposed to 10, 33 or 100 ppm of ethlene oxide (EO). *Toxicology and Applied Pharmacology*, **48**, A84.

Snow, M.H.L. (1976) Embryo growth during the immediate postimplantation period, in *Embryogenesis in Mammals*, Ciba Foundation Symposium N.S. 40, Elsevier, Excerpta Medica, Amsterdam, pp. 53–70.

Snow, M.H.L. (1977) Gastrulation in the mouse: Growth and regionalization of the epiblast. *Journal of Embryology and Experimental Morphology*, **42**, 293–303.

Snow, M.H.L. (1981) Growth and its control in early mammalian development. *British Medical Bulletin*, **37(3)**, 221–226.

Snow, M.H.L. (1986) Control of embryonic growth rate and fetal size in mammals, in *Human Growth*, Vol. l, *Developmental Biology Prenatal Growth* (eds F. Falkner and J.M. Tanner), Plenum Press, New York, pp. 67–82.

Soderwall, A.L., Kent, H.A., Turbyfill, C.L. and Britenbaker, A.L. (1960) Variation in gestation length and litter size of the golden hamster, *Mesocricetus auratus*. *Journal of Gerontology*, **15**, 246–248.

Soller, M., Padeh, B., Wysoki, M. and Ayalon, N. (1969) Cytogenetics of the Saanen goats showing abnormal development of the reproductive tract associated with the dominant gene for polledness. *Cytogenetics*, **8**, 51–67.

Soloff, M.S., Morrison, M.J. and Swartz, T.C. (1972) A comparison of the estrone–estradiol-binding proteins in the plasma of prepubertal and pregnant rats. *Steroids*, **20**, 597–608.

Spencer, R.P. and Coulombe, M.J. (1965) Fetal

weight–gestational age relationship in several species. *Growth*, **29**, 165–171.

Springer, J.E. (1988) Nerve growth factor receptors in the central nervous system. *Experimental Neurology*, **102**, 354–365.

Squiers, C.D., Dickerson, G.E. and Mayer, D.T. (1952) Influence of inbreeding, age and growth rate of sows on sexual maturity, rate of ovulation, fertilization and embryonic survival. *Research Bulletin of the Missouri Agricultural Experimental Station*, No. 494.

Stanley, A.J. and Grumbrech, L.G. (1964) Male pseudohermaphroditism with feminizing testis in the male rat – A sex-linked recessive character. *Program of the Endocrine Society*, p. 40.

Stein, Z., Kline, J., Susser, E., Shrout, P., Warburton, D. and Susser, M. (1980) Maternal age and spontaneous abortion, in *Human Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 107–127.

Sternberg, W.H., Barclay, D.L. and Kloepfer, M.W. (1968) Familial XY gonadal dysgenesis. *New England Journal of Medicine*, **278**, 695–700.

Stevens, C.V., Sparks, S.J. and Powell, J.E. (1970) Levels of estrogens, progestogens and luteinizing hormone during the menstrual cycle of the baboon. *Endocrinology*, **87**, 658–666.

Stevens, V.C. (1976) Antifertility effects from immunisation with intact, subunits and fragments of hCG, in *Pysiological Effects of Innunity against Reproductive Hormones* (eds R.G. Edwards and M.H. Johnson), Cambridge University Press, pp. 249–274.

Stewart, D.L. (1952) The suppression of oestrus in dairy cattle after mating. *Veterinary Record*, **64**, 303–308.

Stewart, H.J., McCann, S.H.E., Barker, P.J., Lee, K.E., Lamming, G.E. and Flint, A.P.F. (1987) Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. *Journal of Endocrinology*, **115**, R13–R15.

Stewart, H.J., Jones, D.S.C., Pascall, J.C., Popkin, R.M. and Flint, A.P.F. (1988) The contribution of recombinant DNA techniques to reproductive biology. *Journal of Reproduction and Fertility*, **83**, 1–57.

Stoner, C.S., Geisert, R.D., Bazer, F.W. and Thatcher, W.W. (1981) Characterization of estrogen patterns in early pregnancy and cyclic gilts. *Journal of Animal Science*, **53**, 308.

Stouffer, R.L., Nixon, W.E. and Hodgen, G.D. (1977) Estrogen inhibition of basal and gonadotropin-stimulated progesterone production by rhesus monkey luteal cells *in vitro*. *Endocrinology*, **101**, 1157–1163.

Stouffer, R.L., Nixon, W.E. and Hodgen, G.D. (1979) Disparate effects of prostaglandins on basal and gonadotrophin-stimulated progesterone production by luteal cells isolated from the rhesus monkeys during the menstrual cycle and pregnancy. *Biology of Reproduction*, Vol. 20, 897–903.

Stouffer, R.L., Ottobre, J.S., Molskness, A. and Zelinski-Wooten, M.B. (1989) The function and regulation of the primate corpus luteum during the fertile menstrual cycle, in *Development of Preimplantation Embryos and their Environment* (eds K. Yoshinaga and T. Mori), Alan R. Liss, New York. pp. 129–142.

Stuart, L.D. and Oehme, F.W. (1982) Environmental factors in bovine and porcine abortion. *Veterinary and Human Toxicology*, **24**, 435–441.

Su, M-Q. and Okita, G.T. (1976) Embryocidal and teratogenic effects of methyl- mercury in mice. *Toxicology and Applied Pharmacology*, **38**, 207–216.

Suchowsky, G.K. and Junkmann, K. (1961) A study of the virilizing effect of progestogens on the female rat fetus. *Endocrinology*, **68**, 341–349.

Sugino, H., Bousfield, G.R., Moore, W.T. and Ward, D.N. (1987) Structural studies on equine glycoprotein hormones. Amino acid sequence of equine chorionic gonadotropin β-subunit. *Journal of Biological Chemistry*, **262**, 8603–8609.

Sullivan, D.J. and Drobeck, H.P. (1966) True hermaphrodism in a rhesus monkey. *Folia Primatologica*, **4**, 309–317.

Sullivan, F.M. and Barlow, S.M. (1985) The relevance for man of animal data on reproductive toxicity of industrial chemicals, in *Prevention of Physical and Mental Congenital Defects*, Part B: Epidemiology, Early Detection and Therapy, and Environmental Factors (ed. M. Marois), Alan R. Liss, New York, pp. 301–305.

Summers, P.M., Wennink, C.J. and Hodges, J.K. (1985) Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). *Journal of Reproduction and Fertility*, **73**, 133–138.

Summers, P.M., Shephard, A.M., Taylor, C.T. and Hearn, J.P. (1987) The effects of cryopreservation and transfer on embryonic development in the common marmsoet monkey, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, **79**, 241–250.

Summers, P.M., Campbell, J.M. and Miller, M.W. (1988) Normal *in vivo* development of marmsoet monkey embryos afetr trophectoderm biopsy. *Human Reproduction* **3**, 389–393.

Sunderman, F.W. Jr., Allpass, P.R., Mitchell, J.M. and Baselt, R.C. (1979) Eye malformations in rats: Induction by prenatal exposure to nickel carbonyl. *Science*, **203**, 550–553.

Surani, M.A.H., Barton, S.C. and Norris, M.L. (1986) Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome. *Cell*, **45**, 127–136.

Susser, M. and Stein, Z. (1980) Prenatal diet and reproductive loss, in *Human Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 183–196.

Sutherland, S.D. and Gorski, R.A. (1972) An evaluation of the inhibition of androgenization of the neonatal female rat brain by barbiturate. *Neuroendocrinology*, **10**, 94–108.

Swanston, I.A., McNatty, K.P. and Baird, D.T. (1977) Concentration of prostaglandin $F_{2\alpha}$ and steroids in the human corpus luteum. *Journal of Endocrinology*, **73**, 115–122.

Swindle, M.M., Craft, C.F., Marriott, B.M., Strandberg, J.D. and Luzarragu, M. (1982) Ascending intrauterine infections in rhesus monkeys. *Journal of the American Veterinary Medical Association*, **181**, 1367–1370.

Tabacova, S., Littinkova, L. and Balavaeva, L. (1978) Carbon disulphide teratogenicity and postnatal effects in rat. *Toxicology Letters*, **2**, 129–133.

Taeusch, H.W. (1975) Glucocorticoid prophylaxis for hyaline membrane disease. *Journal of Pediatrics*, **87**, 617–623.

Takano, K., Yamamura, H., Suzuki, M. and Nishimura, H. (1966) Teratogenic effect of chlormadinone acetate in mice and rabbits. *Proceedings of the Society for Experimental Biology and Medicine*, **121**, 455–457.

Talbert, G.B. (1968) Effect of maternal age on reproductive capacity. *American Journal of Obstetrics and Gynecology*, **102**, 451–477.

Talbert, G.B. (1978) Effect of aging of the ovaries and female gametes on reproductive capacity, in *The Aging Reproductive System* (ed. E.L. Schneider), Raven Press, New York, pp. 59–83.

Talbert, G.B. and Krohn, P.L. (1966) Effect of maternal age on viability of ova and uterine support of pregnancy in mice. *Journal of Reproduction and Fertility*, **11**, 399–406.

Talmadge, K., Boorstein, W.R., Vamuakopolous, N.C., Gethung, M.J. and Fiddes, J.C. (1984a) Only 3 of the 7 hCG beta subunit genes can be expressed in the placenta. *Nucleic Acids Research*, **12**, 8415–8436.

Talmadge, K., Vamvakopoulos, N.C. and Fiddes, J.C. (1984b) Evolution of the genes for the β-subunits of human chorionic gonadotrophin and luteinising hormone. *Nature*, **307**, 37–40.

Talwar, G.P., Dubey, S.K., Salahuddin, M. and Shastri, N. (1976) Kinetics of antibody response in animals injected with processed beta hCG conjugated to tetanus toxoid. *Contraception*, **13**, 153–162.

Tan, G.J.S., Tweedle, R. and Biggs, J.S.G. (1982) Oxytocin may play a role in the control of the human corpus luteum. *Journal of Endocrinology*, **95**, 65–70.

Tanimura, T. (1972) Abnormalities of intrauterine development in nonhuman primates. *Acta Endocrinologica*, Suppl. 71(166), 261–292.

Tarantal, A.F. and Hendrickx, A.G. (1988a) Prenatal growth in the cynomolgus and rhesus macaque (*Macaca fascicularis* and *Macaca mulatta*): A comparison by ultrasonography. *American Journal of Primatology*, **15**, 309–323.

Tarantal, A.F. and Hendrickx, A.G. (1988b) The use of ultrasonography for evaluating pregnancy in macaques in *Proceedings of the Symposium on Non-human Primates – Developmental Biology and Toxicology* (eds D. Neubert, H-J Merker and A.G. Hendrickx), Ueberreuter Wissenschaft, Wien, Berlin, pp. 91–99.

Tarara, R., Enders, A.C., Hendrickx, A.G., Gulamhusein, N., Hodges, J.K., Hearn, J.P., Eley, R.M. and Else, J.G. (1987) Early implantation and embryonic development of the baboon: Stages 5, 6 and 7. *Anatomy and Embryology*, **176**, 267–275.

Tarttelin, M.F. and Gorski, R.A. (1988) Postnatal influence of diethylstilbestrol on the differentiation of the sexually dimorphic nucleus in the rat as is effective as perinatal treatment. *Brain Research*, **456**, 271–274.

Terrill, C.E. (1962) The reproduction of sheep, in *Reproduction in Farm Animals* (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 240–254.

Thatcher, W.W., Knickerbocker, J.J., Bartol, F.F., Bazer, F.W., Roberts, R.M. and Drost, M. (1985) Maternal recognition of pregnancy in relation to the survival of transferred embryos: endocrine aspects. *Theriogenology*, **23**, 129–144.

Thibier, M., El-Hassan, N., Clark, M.R., LeMaire, W.J. and Marsh, J.M. (1980) Inhibition by estradiol of human chorionic gonadotrophin-induced progesterone accumulation in isolated human luteal cells: lack of mediation by prostaglandin F. *Journal of Clinical Endocrinology and Metabolism*, **50**, 590–592.

Thomas, J.A., Felice, P.R., Schein, L.G., Gupta, P.K. and McCafferty, R.E. (1979) Effects of monoethylhexylphthalate (MEHP) on pregnant rabbits and their offspring. *Toxicology and Applied Pharmacology*, **48**, A33.

Thomas, J.P., Dorflinger, L.J. and Behrman, H.R. (1978) Mechanism of the rapid antigonadotropic action of prostaglandins in the cultured luteal cells. *Proceedings of the National Academy of Sciences of the USA*, **75**, 1344–1348.

Thomson, A.M., Billewicz, W.Z. and Hytlen, F.E. (1968) The assessment of fetal growth. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **75**, 903–916.

Thorburn, C.D., Cox, H.I., Currie, W.B., Restall, B.J. and Schneider, W. (1973) Prostaglandin F and progesterone concentrations in the uteroovarian venous plasma of the ewe during the oestrous cycle and early pregnancy. *Journal of Reproduction and Fertility*, Suppl. 18, 151–158.

Tiedemann, K. (1971) Die ultrastruktur des epithels des wolffschen ganges und des ductus deferens beim schafembryo. *Zeitschrift fur Zellforschung und Mikroskopische Anatomie*, **13**, 230–248.

Tompkins, E.C., Heidenreich, C.J. and Stob, M. (1967) Effect of post-breeding thermal stress on embryonic mortality in swine. *Journal of Animal Science*, **26**, 377–380.

Toran-Allerand, C.D. (1984a) Gonadal hormones and brain development: implications for the genesis of sexual differentiation. *Annals of the New York Academy of Sciences*, **435**, 101–110.

Toran-Allerand, C.D. (1984b) On the genesis of sexual differentiation of the central nervous system: morphogenetic consequences of steroidal exposure and possible role of alpha-fetoprotein, in *Progress in Brain Research*, Vol. 61 (eds G.J. deVries, J.P.C. deBruin, H.B.M. Uylings and M.A. Corner), Elsevier, Amsterdam, pp. 63–97.

Torrey, T.W. (1954) The early development of the human nephros. *Contributions to Embryology Carnegie Institute*, **35**, 175–197.

Tourniaire, J., Audi- Parera, L., Loras, B., Blum, J., Castelnovo, P. and Forest, M.G. (1976) Male pseudohermaphroditism with hypertension due to a l7a-hydroxylase deficiency. *Clinical Endocrinology*, **5**, 53–61.

Tran, D., Meusy-Dessolle, N. and Josso, N. (1977) AntiMullerian hormone is a functional marker of foetal Sertoli cells. *Nature*, **269**, 411–412.

Trasler, D.G. (1965) Aspirin-induced cleft lip and other malformations in mice. *Lancet*, **1**, 606.

Tuchmann-Duplessis, H. (1965) Design and interpretation of teratogenic tests, in *Embryopathic activity of drugs* (eds J.M. Robson F.M. Sullivan and R.L. Smill), Little, Brown & Co. Boston, pp. 56–93.

Tuchmann-Duplessis, H. (1970) Animal species and drug- induced teratogenicity. *Proceedings of the European Society for the Study of Drug Toxicity*, **11**, 33–49.

Turbow, M.M. and Chamberlain, J.G. (1968) Direct effects of 6-aminonicotinamide on the developing rat embryo *in vitro* and *in vivo*. *Teratology*, **1**, 103–108.

Tureck, R.W., Mastroianni, L., Blasco, L. and Strauss, J.F. (1982) Inhibition of human granulosa cell progesterone secretion by a gonadotropin-releasing hormone agonist. *Journal of Clinical Endocrinology and Metabolism*, **54**, 1078–1080.

Tze, W.J. and Lee, M. (1975) Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats. *Nature*, **257**, 479–480.

Ulberg, L.C. (1958) The influence of high temperature on reproduction. *Journal of Heredity*, **49**, 62–64.

Ulberg, L.C. (1962) The reproduction of cattle, in *Reproduction in Farm Animals* (ed E.S.E. Hafez), Lea and Febiger, Philadelphia, pp. 229–239.

Ulfelder, H. and Robboy, S.J. (1976) The embryonic development of the human vagina. *American Journal of Obstetrics and Gynecology*, **126**, 769–776.

Ullrey, D.E., Spraque, J.I. Becker, D.E. and Miller, E.R. (1965) Growth of the swine fetus. *Journal of Animal Science*, **24**, 711–717.

Upadhyay, S., Luciana, J.M. and Zamboni, L. (1979) The role of the mesonephros in the development of indifferent gonads and ovaries of the mouse. *Annales de Biologie Animale, Biochimie et Biophysique*, **19**, 1179–1196.

Valenzuela, G., Balmaceda, J.P., Harper, M.J.K. and Asch, R.H. (1983) Platelets bind to rhesus monkey corpora lutea and stimulate its prostaglandin synthesis. *Fertility and Sterility*, **39**, 370–373.

Vallet, J.L., Bazer, F.W., Fliss, M.F.V. and

Thatcher, W.W. (1988) Effect of ovine conceptus proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandins F-2α and E of 13,14-dihydro-15-keto prostaglandin F-2α in cyclic sheep. *Journal of Reproduction and Fertility*, **84**, 493–504.

Vandeberg, J.L. (1983) Developmental aspects of X chromosome inactivation in eutherian and metatherian mammals. *Journal of Experimental Zoology*, **228**, 271–286.

Vandevelde, J.E. (1965) True hermaphroditism in a dog. *Canadian Veterinary Journal*, **6**, 241–242.

Van de Wiele, R.L., Bogumil, J., Dyrenfurth, I., Ferin, M., Jewelewicz, R., Warren, M., Rizkallah, T. and Mikhail, G. (1970) Mechanisms regulating the menstrual cycle in women. *Recent Progress in Hormone Research*, **26**, 63–103.

Van Niekerk, W.A. (1974) *True Hermaphroditism, Clinical, Morphologic and Cytogenetic Aspects*, Harper and Row, New York.

Van Niekerk, W.A. (1981) True hermaphroditism, in (ed. N. Josso), *The Intersex Child*, Karger, Basle, pp. 80–99.

Vigier, B., Prepin, J. and Jost, A. (1976) Chronologie du développement de l'appareil génital du foetus de veau. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale*, **65**, 77–102.

Virdis, R., Saenger, P., Senior, B. and New, M.I. (1978) Endocrine studies in a pubertal male pseudohermaphrodite with 17-ketosteroid reductase deficiency. *Acta Endocrinologica*, **87**, 212–224.

Voigt, W. and Hsia, S.L. (1973) Further studies on testosterone 5-alpha-reductase of human skin: structural features of steroid inhibitors. *Journal of Biological Chemistry*, **248**, 4280–4285.

Vorherr, H., Messer, R.H., Vorherr, M.F., Jordan, S.W. and Kornfeld, M. (1979) Teratogenesis and carcinogenesis in rat offspring after transplacental and transmammary exposure to diethylstilbestrol. *Biochemical Pharmacology*, **28**, 1865–1877.

Wachtel, S.S. (1986) Sex determination. *New England Journal of Medicine*, **314**, 61–62.

Wachtel, S.S. and Koo, G.C. (1981) H-Y antigen in gonadal differentiation, in *Mechanisms of Sex Differentiation in Animals and Man*, (eds C.R. Austin and R.G. Edwards), Academic Press, New York, pp. 255–299.

Wachtel, S.S., Koo, G.C., Zuckerman, E.E., Hammerling, U., Scheid, M.P. and Boyse, E.A. (1974) Serological crossreactivity between H-Y (male) antigens of mouse and man. *Proceedings of the National Academy of Sciences of the USA*, **71**, 1215–1218.

Wachtel, S.S., Koo, G.C. and Boyse, E.A. (1975a) Evolutionary conservation of H-Y ('male') antigen. *Nature*, **254**, 270–272.

Wachtel, S.S., Ohno, S., Koo, G.C. and Boyse, E.A. (1975b) Possible role for H-Y antigen in the primary determination of sex. *Nature*, **257**, 235–236.

Wachtel, S.S., Ohno, S., Koo, G.C. and Boyse, E.A. (1975c) Possible role for H-Y antigen in the primary determination of sex. *Nature*, **257**, 235–236.

Wachtel, S.S., Koo, G.C., Breg, W.R., Thaler, H.T., Dillard, G.M., Rosenthal, I.M., Dosik, H., Gerald, P.S., Saenger, P., New, M., Lieber, E. and Miller, O.J. (1976) Serologic detection of a Y-linked gene in XX males and XX true hermaphrodites. *New England Journal of Medicine*, **295**, 750–754.

Wachtel, S.S., Koo, G.C., de le Chapelle, A., Kallio, H., Heyman, J.M. and Miller, O.J. (1980) H-Y antigen in 46, XY gonadal dysgenesis. *Human Genetics*, **54**, 25–30.

van Wagenen, G. and Catchpole, H.R. (1965) Growth of the fetus and placenta of the monkey (*Macaca mulatta*). *American Journal of Physical Anthropology*, **23**, 22–33.

van Wagenen, G. and Morse, A.H. (1944) Estrogen tolerance in pregnancy. *Yale Journal of Biology and Medicine*, **17**, 301–309.

Wai-Sum, O., Short, R.V., Renfree, M.B. and Shaw, G. (1988) Primary genetic control of somatic sexual differentiation in a mammal. *Nature*, **331**, 716–717.

Walker, B.E. (1984) Transplacental exposure to diethylstilbestrol, in *Issues and Reviews in Teratology* (ed. H. Kalter), Plenum Press, New York pp. 157–187.

Warburton, D. (1987) Chromosomal causes of fetal death. *Clinical Obstetrics and Gynecology*, **30**, 268–277.

Warburton, D. and Fraser, F.C. (1964) Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. *American Journal of Human Genetics*, **16**, 1–25.

Warburton, D., Stein, Z., Kline, J. and Susser, M. (1980) Chromosome abnormalities in spontaneous abortions: data from the New York study, in *Human Embryonic and Fetal Death* (eds

I.H. Porter and E.B. Hook), Academic Press, New York, pp. 261–287.

Ward, I. (1969) Differential effect of pre- and postnatal androgen on the sexual behavior of intact and spayed female rats. *Hormones and Behavior*, **1**, 25–36.

Ward, I.L. and Renz, F.J. (1972) Consequences of perinatal hormone manipulation on the adult sexual behavior of female rats. *Journal of Comparative Physiology and Psychology*, **78**, 349–355.

Wartenberg, H. (1979) Der mesonephros und die gonadenentwicklung. *Verhandlungen der Anatomischen Gesellschaft*, **73**, 385–401.

Wartenberg, H. (1981) Differentiation and development of the testes, in *The Testis* (eds H. Burger and D. M. de Kretser), Raven Press, New York, pp. 39–80.

Wartenberg, H. (1985) Origin of gonadal blastemal cells in mammalian gonadogenesis. *Archives D Anatomie Microscopique et de Morphologie Experimentale*, **74(1)**, 60–63.

Watanabe, G., Yoshida, S. and Rose, R. (1968) Teratogenic effect of benzol in pregnant mice. *Congenital Anomalies*, **8**, 45–46.

Wathes, D.C. and Swann, R. (1982) Is oxcytocin an ovarian hormone? *Nature*, **297**, 225–227.

Wathes, D.C., Pickering, B.T., Swann, R.W., Ported, D.G., Hull, M.G.R. and Drife, J.O. (1982) Neurohypophyseal hormones in the human ovary. *Lancet*, **2**, 410–412.

Webley, G.E. and Hearn, J.P. (1987) Local production of progesterone by the corpus luteum of the marmoset monkey in response to perfusion with chorionic gonadotrophin and melatonin *in vivo*. *Journal of Endocrinology*, **112**, 449–457.

Webley, G.E., Luck, M.R. and Hearn, J.P. (1988) Stimulation of progesterone secretion by cultured human granulosa cells with melatonin and catecholamines. *Journal of Reproduction and Fertility*, **84**, 669–677.

Webley, G.E., Richardson, M.C., Summers, P.M., Given, A. and Hearn, J.P. (1989a) Changing responsiveness of luteal cells of the marmoset monkey (*Callithrix jacchus*) to luteotrophic and luteolytic agents during normal and conception cycles. *Journal of Reproduction and Fertility*, **87**, 301–310.

Webley, G.E., Richardson, M.C. and Hearn, J.P. (1989b) Disparate effects of cloprostenol *in vivo* and *in vitro* in the marmoset monkey indicate a mechanism of action for the control of luteal function. *Journal of Endocrinology*, Suppl. 121, 228.

Webster, W.S., Lipson, A.H., Sulik, K.K. (1988) Interference with gastrulation during the third week of pregnancy as a cause of some facial abnormalities and CNS defects. *American Journal of Medical Genetics*, **31**, 505–512.

Weil, R.J. and Tupper, C. (1960) Personality life situation and communication: a study of habitual abortion. *Psychosomatic Medicine*, **22**, 448–455.

Weiland, N.G. and Barraclough, C.A. (1984) Neonatal castration of male and female rats affects hypothalamic and pituitary estrogen nuclear and progestin cytosol receptors. *Biology of Reproduction*, **31**, 950–958.

Weisz, J. and Ward, I.L. (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology*, **106**, 306–316.

Welsch, F. (ed.) (1987) *Approaches to Elucidate Mechanisms in Teratogenesis*, Hemisphere Publishing, Washington.

Wentz, A.C. and Jones, G.S. (1973) Transient luteolytic effect of prostaglandin $F_{2\alpha}$ in the human. *Obstetrics and Gynecology*, **42**, 172–181.

Westfahl, P.K. and Kling, O.R. (1982) Relationship of estradiol to luteal function in the cycling baboon. *Endocrinology*, **110**, 64–69.

Westfahl, P.K. and Resko, J.A. (1983) The affects of clomiphene in luteal function in the nonpregnant cynomologus macaque. *Biology of Reproduction*, **29**, 963–969.

Wharton, L.R. Jr and Scott, R.B. (1964) Experimental production of genital lesions with norethindrone. *American Journal of Obstetrics and Gynecology*, **89**, 701–715.

Whittingham, D.G. (1971) Survival of mouse embryos after freezing and thawing. *Nature*, **233**, 125–126.

Wilk, A.L. (1969) Production of fetal rat malformations by norchoreyelizine and chlorcylizine after intrauterine application. *Teratology*, **2**, 55–66.

Wilks, J.W. (1983) The effect of oxytocin on the corpus luteum of the monkey. *Contraception*, **28**, 267–272.

Wilks, J.W. and Nobile, A.S. (1983) Steroidogenic responsiveness of the monkey corpus luteum to exogenous chorionic gonadotrophin. *Endocrinology*, **112**, 1256–1266.

Williams, M.T., Roth, M.S., Marsh, J.M. and Lemaire, W.J. (1979) Inhibition of human chorionic gonadotrophin induced progesterone syn-

thesis by estradiol in isolated human luteal cells. *Journal of Clinical Endocrinology and Metabolism*, **48**, 437–440.

Wilmut, I. (1972) The effect of cooling rate, cryoprotective agent and stage of development on survival of mouse embryos during freezing and thawing. *Life Sciences*, **11**, 1071–1079.

Wilmut, I., Sales, D.I. and Ashworth, C.J. (1986) Maternal and embryonic factors associated with prenatal loss in mammals. *Journal of Reproduction and Fertility*, **76**, 851–864.

Wilson, J.D. (1989) Sexual differentiation of the gonads and of the reproductive tract. *Biology of the Neonate*, **55**, 322–330.

Wilson, J.D. and Lasnitzki, I. (1971) Dihydrotestosterone formation in fetal tissues of the rabbit and rat. *Endocrinology*, **89**, 659–668.

Wilson, J.D. and Siiteri, P.K. (1973) Developmental pattern of testosterone synthesis in the fetal gonad of the rabbit. *Endocrinology*, **92**, 1182–1191.

Wilson, J.D., Griffin, J.E., Leshin, M. and George, F.W. (1981) Role of gonadal hormones in development of sexual phenotypes. *Human Genetics*, **58**, 78–84.

Wilson, J.G. (1959) Experimental studies on congenital malformations. *Journal of Chronic Diseases*, **10**, 111–130.

Wilson, J.G. (1972) Abnormalities of intrauterine development in nonhuman primates. *Acta Endocrinologica*, Suppl. 71, 261–292.

Wilson, J.G. (1973) *Environment and birth defects*, Academic Press, New York, pp.

Wilson, J.G. (1978) Rats (developmental abnormalities), in *Pathology of Laboratory Animals*, Vol. 2 (eds K. Benirschke, F.M. Garner and T.J. Jones), Springer-Verlag, New York, pp. 184–1847.

Wilson, J.G. (1980) Environmental effects on intrauterine death in animals, in *Human Embryonic and Fetal Death* (eds I.H. Porter and E.B. Hook), Academic Press, New York, pp. 19–27.

Wilson, J.G. and Brent, R.L. (1981) Are female sex hormones teratogenic? *American Journal of Obstetrics and Gynecology*, **141**, 567–580.

Wilson, J.G. and Fraser, F.C. (1977) *Handbook of Teratology*, Vol. 1, General Principles and Etiology. Plenum Press, New York, pp. 476.

Wilson, R.F., Nalbandov, A.V. and Krider, J.L. (1949) Impaired fertility in female swine. *Journal of Animal Science*, **8**, 558–568.

Wilson, R.S. (1976) Concordance in physical growth for monozygotic and dizygotic twins. *Annals of Human Biology*, **3**, 1–10.

Wilson, T.M., Therrien, A. and Harkness, J.E. (1986) Sperm counts and reproductive tract lesions in male syrian hamsters exposed in utero to diethylstilbestrol. *Laboratory Animal Science*, **36**, 41–44.

Wimsatt, W.A. (1975) Some comparative aspects of implantation. *Biology of Reproduction*, Vol. 12, 1–40.

Wiseman, R.A. and Dodds-Smith, I.C. (1984) Cardiovascular birth defects and some antenatal exposure to female sex hormones: A reevaluation of some base data. *Teratology*, **30**, 359–370.

Wislocki, G.B. and Streeter, G.L. (1938) On the placentation of the macaque (*Macaca mulatta*) from the time of implantation until the formation of the definitive placenta. *Contributions to Embryology, Carnegie Institute*, **27**, 1–66.

Witschi, E. (1963) Embryology of the ovary, in *The ovary* (eds H.G. Grady and D.E. Smith), Williams & Wilkins, Baltimore, pp. 1–10.

Witschi, E. (1970) Development and differentiation of the uterus, in *Prenatal Life* (ed. H.C. Mack), Wayne State University Press, Detroit pp. 11–35.

Wolf, H., Sabata, V., Frerichs, H. and Stubbe, P. (1969) Evidence for the impermeability of the human placenta for insulin. *Hormone and Metabolic Research*, **1**, 274–275.

Wolf, R.C., O'Connor, R.F. and Robinson, J.A. (1977) Cyclic changes in plasma progestins and estrogens in squirrel monkeys. *Biology of Reproduction*, **17**, 228–231.

Wolf, U. (1979) XY gonadal dysgenesis and the H-Y antigen. *Human Genetics*, **47**, 269–277.

World Health Organization (WHO): *Guidelines of Evaluation of Drugs for Use in Man* (1975), World Health Organization Technical Department, Serial No. 563, Geneva.

Wright, L.J., Feinstein, A., Heap, R.B., Saunders, J.C., Bennett, R.C., Wang, M.Y. (1982) Antiprogesterone monoclonal antibody locks implantation in mice. *Nature*, **29**, 415–417.

Yeko, T.R., Khan-Dawood and Yusoff Dawood, M. (1989) Human corpus luteum: luteinizing hormone and chorionic gonadotropin receptors during the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, **68**, 529–534.

Yoshinaga, K., Hess, D.L., Hendrickx, A.G. and Zamboni, L. (1988) The development of the sexually indifferent gonad in the Prosimian *Galago*

crassicaudatus crassicaudatus. *American Journal of Anatomy*, **181**, 89–105.

Young, N.A. (1976) Chickenpox, measles and mumps in *Infectious Disease of the Fetus and Newborn Infant*, (eds J.S. Remington and J.O. Klein), W.B. Saunders, Philadelphia, pp. 521–587.

Zachmann, M., Wender, E.A. and Prader, A. (1982) Two types of male pseudohermaphroditism due to 17,20-desmolase deficiency. *Journal of Endocrinology and Metabolism*, **55**, 487–490.

Zachmann, M., Vollmin, J.A., Hamilton, W. and Prader, A. (1972) Steroid 17,20-desmolase deficiency: a new cause of male pseudohermaphroditism. *Clinical Endocrinology*, **1**, 369–385.

Zamboni, L. and Upadhyay, S. (1983) Germ cell differentiation in mouse adrenal glands. *Journal of Experimental Zoology*, **228**, 173–193.

Zamboni, L., Bezard, J. and Mauleonm, P. (1979) The role of the mesonephros in the development of the sheep fetal ovary. *Annals of Biological Animal Biochemistry and Biophysics*, **19**, 1153–1178.

Zenzes, M.T., Wolf, U. and Engel, W. (1978a) Orgnaization *in vitro* of ovarian cells into testicular structures. *Human Genetics*, **44**, 333–338.

Zenzes, M.T., Wolf, U., Gunther, E. and Engel, W. (1978b) Studies on the function of H-Y antigen: dissociation and reorganization experiments on rat gonadal tissue. *Cytogenetics and Cell Genetics*, **20**, 365–372.

diZerega, G.S. and Hodgen, G.D. (1980) Changing functional status of the monkey corpus luteum. *Biology of Reproduction*, **23**, 253–263.

Zighelboim, I., Mackelt, G.A., Teppa, P., Perera, J.R., deTeppa, D.G. and Maneiro, P. (1968) Reproductive wastage and *Toxoplasma* antibodies. *American Journal of Obstetrics and Gynecology*, **101**, 839–849.

Zondek, L.H. and Zondek, T. (1965) The secretory activity of the maturing epididymis compared with maturational changes in other reproductive organs of the foetus, infant and child. *Acta Paediatricia Scandinavica*, **54**, 295–305.

ENDOCRINOLOGY OF PREGNANCY, PARTURITION AND LACTATION IN MARSUPIALS

7

Marilyn B. Renfree

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7.1 INTRODUCTION

The earliest mammals were all small, nocturnal insectivorous creatures, and it appears that the basic mode of mammalian reproduction remained unchanged from the time of the origin of mammals in the late Triassic (about 200 million years ago) until the dispersal of the Gondwanan continents in the middle Cretaceous (95–110 million years ago). This meant that there was no land faunal communication between Africa and the Antarctic–Australia–India complex except via South America after the earliest Cretaceous (Lillegraven *et al.*, 1979). The major adaptive radiations of the advanced therian mammals occurred during the middle Cretaceous, so that by the late Cretaceous (75–80 million years ago) the Eutheria and Metatheria were already distinct and largely geographically isolated (Archer, 1984; Tyndale-Biscoe and Renfree, 1987). Whilst marsupials are true mammals in every sense, and in endocrine terms have the same range of hormones, these hormones are sometimes used in different ways (Amoroso *et al.*, 1980) – for example, prolactin inhibits the marsupial corpus luteum, whereas it is luteotrophic in some eutherian mammals. This communality of hormones but diversity of actions between the mammalian groups clearly demonstrates the validity of Medawar's (1953) aphorism that it is not hormones that have evolved, but rather the uses to which they are put.

There are about 250 living species of marsupials representing about 6% of the mammalian fauna (Kirsch, 1977), distributed widely in South America, Australia and New Guinea, but we have detailed endocrine knowledge of only a small number of these (Tyndale-Biscoe and Renfree, 1987). Most information on marsupial reproduction in the past has been based on wild-caught specimens, but the establishment of self-sustaining laboratory colonies of several species now makes possible more conventional laboratory studies (Renfree and Tyndale-Biscoe, 1978; Tyndale-Biscoe and Janssens, 1988; Hinds *et al.*, 1990a). Each family grouping of marsupials shares a common and distinctive reproductive pattern, with each species displaying modifications of the basic pattern in relation to its own constraints of size, diet and habitat (Tyndale-Biscoe, 1984; Lee and Cockburn, 1985). The majority of marsupials are polyoestrous, and, compared with eutherian mammals, have relatively uniform gestation periods that range between 12 and 36 days. This means that pregnancy is remarkably short even for the largest marsupials but since they have such a prolonged and sophisticated lactation, the young marsupial when weaned is as developed as any eutherian at the same stage.

There has recently been a great burgeoning of knowledge about marsupial reproduction, and the whole of marsupial reproductive physiology and development has been reviewed (Tyndale-Biscoe and Renfree, 1987). Several major reviews on various aspects of reproduction have also been published (Tyndale-Biscoe *et al.*, 1974, 1986; Renfree, 1982, 1983; Tyndale-Biscoe, 1984, 1986, 1989; Renfree *et al.*, 1987, 1989; Renfree and Short, 1988; Renfree 1992, 1993).

Two decades ago there was scarcely any knowledge about the endocrinology of marsupials, although it was recognized that the corpus luteum is of central importance, and that progesterone is present in red kangaroo, wallaroo and opossum corpora lutea (Lindner and Sharman, 1966; Cook and Nalbandov, 1968; and Short and Sharman, (unpublished observations) cited in J.G. Smith *et al.*, 1969). Plasma progesterone was measured subsequently in the possum (Thorburn *et al.*, 1971) and the tammar (Lemon, 1972). Today, most is known of the reproductive endocrinology of the tammar wallaby, *Macropus eugenii*, in which all of the major reproductive hormones have been

studied. In this chapter, endocrinology will provide the linking theme between pregnancy, parturition and lactation. Details of marsupial breeding biology, anatomy, development, male reproduction, seasonal breeding and the evolution of reproduction can all be found in Tyndale-Biscoe and Renfree (1987) and the excellent chapter in Volume 1 of this edition by Tyndale-Biscoe (1984).

7.2 PATTERNS OF REPRODUCTION

Most marsupials are seasonal breeders and produce relatively small young, ranging from the honey possum, *Tarsipes rostratus*, which delivers the smallest neonate weighing less than 5 mg at birth (Renfree, 1980a), to the largest, the kangaroos, which deliver neonates weighing around 800 mg. Although the maternal investment in **pregnancy** is very small and much less than that of eutherian mammals, this is more than made up for during the long **lactation**. Marsupials may be monoestrous or polyoestrous, and in most species lactation controls reproduction by preventing either ovulation or post-blastocyst development. In seasonally breeding marsupials the animals may become either anoestrous or reproductively quiescent at the end of the season, regardless of whether lactation continues. The shortest gestation period is 9.5 to 12 days in *Sminthopsis macroura* (Gemmell and Selwood, 1993), followed by 12½ days, as found in bandicoots (Lyne, 1974) and opossums (Hartman, 1923, 1928). The longest gestation periods so far described are amongst kangaroos, being 36 days in the grey kangaroo, *Macropus giganteus* (reviewed in Tyndale-Biscoe, 1984), and 44 days in Matchie's tree kangaroo, *Dendrolagus matschiei* (L.R. Collins, personal communication). In the macropodids pregnancy may be extended even further by embryonic diapause. Detailed tables of the breeding biology of marsupials have been provided extensively in Tyndale-Biscoe and Renfree (1987), and a summary table showing the gestation periods of some commonly studied species is given here (Table 7.1).

The oestrous cycle of marsupials is very similar to that of the Eutheria. The histological changes in the uterus and ovary during the follicular and luteal phases resemble those seen in many other mammals (see Pilton and Sharman, 1962; Sharman and Berger, 1969). The steroid hormones and gonadotrophins involved in reproduction in eutherian mammals have been measured in marsupials, though as we will see later particular features of their functions differ (Tyndale-Biscoe and Renfree, 1987). Ovulation is spontaneous in all marsupials and in most species occurs within 2 days of oestrus.

The marsupial oestrous cycle varies in mean length from 22 to 42 days, the average being 28 days (Tyndale-Biscoe, 1973) (see Table 7.1). However, in some didelphids, such as the woolly opossum, *Caluromys philander*, which has a cycle of 38 days (Perret and Atrementowicz, 1989), it may range up to 40 days (Godfrey, 1975; Fadem and Rayve, 1985). The life of the corpus luteum is not prolonged by the presence of a conceptus, although in the Peramelidae (bandicoots) the corpus luteum persists during lactation (Hughes, 1965; Gemmell, 1979). Because pregnancy is shorter than the oestrous cycle in all except the swamp wallaby, *Wallabia bicolor*, the histological changes of pregnancy and the oestrous cycle are similar (Figure 7.1), and the young are born, depending on the species, at different stages of the luteal and follicular cycle (Figure 7.2).

The timing of birth and oestrus can be altered experimentally, suggesting that gestation and oestrous cycle activity need not be closely linked (Clark, 1968). In the American opossums *Didelphis virginiana* and *Marmosa mitis*, as in many monovular and polyovular Australian species, the young are born towards the end of the luteal phase, and the mother's return to oestrus is suppressed by the sucking stimulus. By contrast, in the

Table 7.1 Duration of the oestrous cycle, gestation and litter size and occurrence of embryonic diapause for a few selected species of marsupial. (Condensed from Tyndale-Biscoe and Renfree, 1987)

Species	*Oestrous cycle (days)*	*Gestation (days)*	*Diapause*	*RPY to birth (days)*	*Litter size*	*Birth weight (mg)*
Didelphidae						
Didelphis virginiana	25.5	12.5	N	26	3–13	130
Monodelphis domestica	32.3	13.5	N	–	3–14	100
Dasyuridae						
Dasyurus viverrinus	37	19	N	?	5.8	12.5
Antechinus stuartii	Mon-oestrous	27	Y	Never		16
Tarsipedidae						
Tarsipes rostratus			Y	?(>90)	2.4	5
Phalangeridae						
Trichosurus vulpecula	25.7	17.5	N	26	1	200
Perameliidae						
Perameles nasuta	21 (10–34)	12.5	N	–	2.4	287
Isoodon macrourus	26.5 (9–34)	12.5	N	–	3.4	180
Macropodidae						
Potorous tridactylus	42	38	Y	29	1	330
Bettongia gaimardi	23.2	21.1	Y	18	1	300
Macropus giganteus	45.6±9.8	36.4±1.6	Y	28–32	1	740
Macropus fuliginosus	34.9±4.4	30.6±2.6	N	Never	1	828
Setonix brachyurus	28	27	Y	25	1	
Macropus eugenii	30.6	29.3	Y	26.4±1	1	440
Macropus rufogriseus	31.9	29.4	Y	27.8	1	450
Macropus rufus	34.8±0.6	33.2±0.2	Y	31.3±0.4	1	817

N, no; Y, yes; RPY, removal of pouch young.

macropodids, the single-pouch young reaches the pouch at the end of the pro-oestrous phase, and ovulation and fertilization occur post-partum (Figure 7.2). In most of the Macropodoidea, the fertilized egg enters a dormant phase at the blastocyst stage at about the eighth day post coitum, and during this period of embryonic diapause both the blastocyst and the corpus luteum remain quiescent as long as the suckling pouch young is present. If the pouch young is removed (RPY), the quiescent blastocyst reactivates and pregnancy ensues. In *M. eugenii*, and in Bennett's wallaby, *M. rufogriseus rufogriseus*, photoperiodic influences maintain the diapause in the second half of the year after the winter solstice (see Figure 7.3), and reactivation occurs spontaneously after the longest day.

Marsupial reproductive strategies have

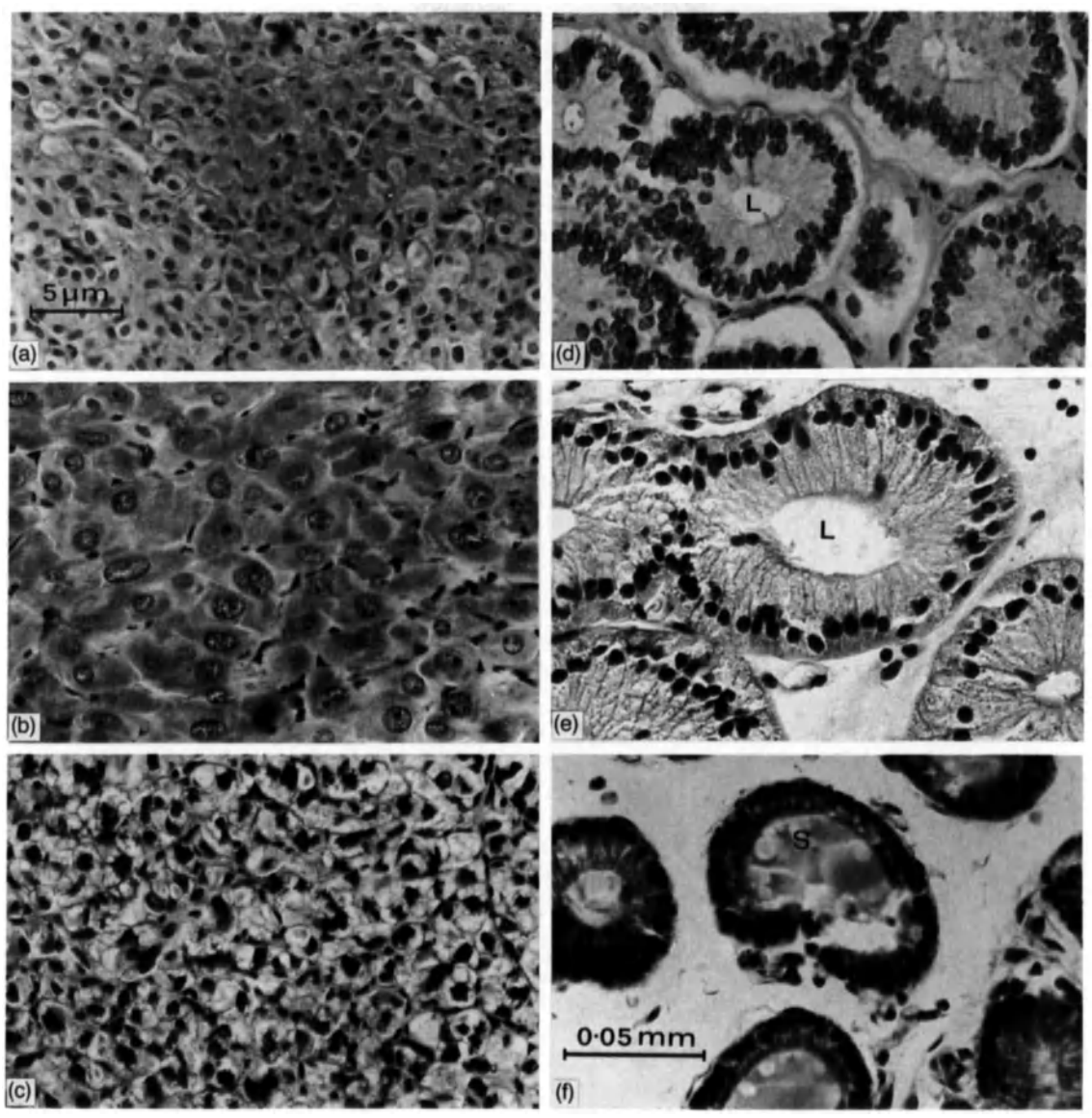


Figure 7.1 Histology of the corpus luteum and uterine endometrium of the tammar wallaby during gestation after removal of the pouch young (RPY). (a–c) Sections of the corpus luteum (all at same magnification: scale bar 5 µm) showing the hypertrophy and hyperplasia of luteal cells after RPY, and their subsequent degeneration with pyknotic nuclei by day 25 of the 26.5 days' gestation. (a) Day 2 RPY; (b) day 17 RPY; (c) day 25 RPY. (d–f) Sections of the endometrium (all at same magnification: scale bar 0.05 mm). (d) Uterine glands in endometrium on day 0 RPY. (e) Uterine glands on day 17 RPY and (f) on day 27 RPY. At mid-pregnancy, the uterine glands are noticeably expanded, with tall columnar epithelial cells surrounding the enlarged gland lumina (L). By day 27, immediately after birth, the cells have darkly staining, basal nuclei although the gland lumina contain residual secretion.

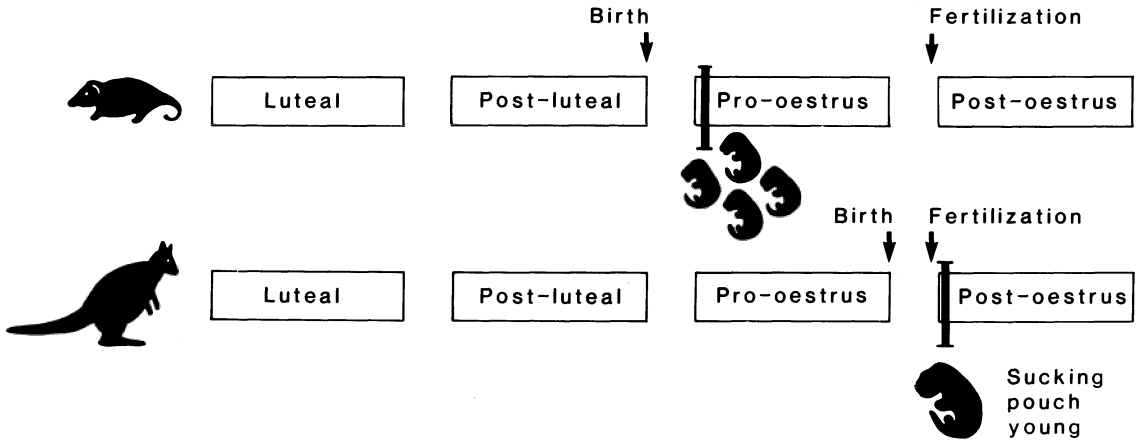


Figure 7.2 The relationship of marsupial oestrous cycles to pregnancy. In species such as the polyovular American opossum (*Didelphis virginiana*) (top) belonging to group 1 reproductive pattern (see text), birth occurs toward the end of the uterine secretory phase, and the presence of the numerous young in the pouch prevents the pro-oestrus phase (dark bar) and the development of any new follicles. Should the sucking young be lost, animals return to oestrus about 7 days later, with the subsequent pregnancy taking only 12.5 days. In the macropodids, such as the monovular tammar wallaby (*Macropus eugenii*) (bottom), with the group 3 reproductive pattern, gestation occupies the whole length of the oestrous cycle, and ovulation occurs within a day of the birth of the single young. Sucking of the pouch young prevents development of the new conceptus beyond the blastocyst stage (dark bar) (arrows show the relative times of ovulation and fertilization). (Redrawn from Renfree, 1980b.)

been divided into four categories by Tyndale-Biscoe and Renfree (1987). These are:

Group 1: Polyovular, polyoestrous species in which the gestation period is considerably shorter than the oestrous cycle and coincides with the luteal phase; post-partum oestrus and ovulation are suppressed during lactation. This pattern seems to be common to the possum families, Petauridae, Phalangeridae, and to the majority of the carnivorous groups, the Polyprotodonta as well. Tyndale-Biscoe (1984) concludes that it represents the basic marsupial pattern from which the other patterns have been derived. The evidence from carnivorous Dasyuridae suggests that the monoestrous pattern of the brown marsupial mouse *Antechinus* and *Phascogale* has been derived from a polyoestrous pattern as special adaptations of seasonal breeding.

Group 2: Polyoestrous, polyovular marsupials with an ultra-short gestation occupying less than the luteal phase, which is prolonged into lactation. In addition there is a well-developed chorioallantoic placenta (the bandicoot families Peramelidae, Thylacomyidae).

Group 3: Monovular, polyoestrous species in which the gestation period is almost the same length as the cycle and extends into the follicular phase so that post-partum oestrus and ovulation occur. During lactation further development is arrested, and if fertilization occurs the embryo remains as a unilaminar blastocyst of about 100 cells in embryonic diapause. Most of the kangaroo and wallaby family Macropodidae share this pattern, with the exceptions of *M. giganteus, M. fuliginosis, M. parma, M. parryi* and *D. matschiei*, which follow an essentially group 1 pattern. In two species, *M. eugenii* and *M. rufogriseus rufogriseus*, a photoperiodic seasonal control of diapause has been superimposed on the lactational one after the winter solstice.

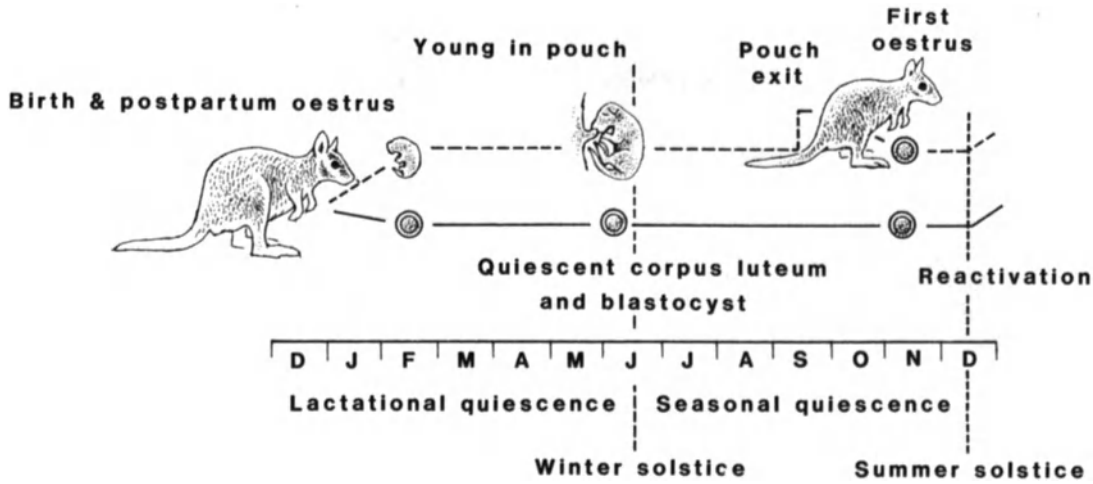


Figure 7.3 The annual cycle of reproduction in the tammar, in the southern hemisphere. Most females give birth towards the end of January or early February, a month or so after the summer solstice on December 21/22. Within 8 h of birth the females come into oestrus, mate and the fertilized egg develops into a 100-cell blastocyst. The blastocyst remains in diapause until the summer solstice. The pouch young leaves the pouch around October, and if it is a female may conceive shortly thereafter. The conceptus immediately enters diapause and the female does not go into active pregnancy until the following solstice. (From Tyndale-Biscoe and Renfree, 1987, with permission.)

Group 4: Polyoestrous, polyovular, with a very prolonged pre-luteal phase and gestation, which includes a long period of embryonic diapause after a post-partum oestrus. It is unclear as to whether diapause is controlled by lactation or is obligatory. The small possum families Burramyidae (except *Burramys* which has the group 1 pattern), Acrobatidae and Tarsipedidae fall into this group.

7.3 EARLY PREGNANCY: OVULATION, CLEAVAGE AND THE EARLY BLASTOCYST

Folliculogenesis overlaps with the last half of pregnancy in macropodid marsupials, and the sequence of events is best known for the tammar. The follicular cycle begins around mid-pregnancy, when progesterone concentration is high. Graafian follicles are evident in the ovary from about day 23 of pregnancy after removal of the pouch young (RPY), and reach their maximum size at post-partum oestrus (Harder *et al.*, 1984). The concentration of progesterone in the peripheral circulation and in the uterine branch of the ovarian vein draining the corpus luteum falls precipitously at birth, or more slowly after a non-pregnant cycle (Hinds and Tyndale-Biscoe, 1982a; Towers *et al.*, 1986), but oestradiol 17β peaks in the peripheral circulation and in the ovarian branch of the ovarian vein draining the ovary with the Graafian follicle (Harder *et al.*, 1984; Shaw and Renfree, 1984) about 8–10 h post-partum (Figures 7.4 and 7.5).

This peak of oestradiol coincides with behavioural oestrus, and removal of the Graafian follicle prevents oestrus and the luteinizing hormone (LH) pulse which follows the oestradiol rise by about 7 h (Harder *et al.*, 1985; Horn *et al.*, 1985). Similarly, interference with the LH pulse by immunization prevents folliculogenesis and the ovulation which usually occurs about 30 h post-partum (Short *et al.*, 1985). In the brush possum oestradiol is measurable on the day of oestrus but not at other times (Curlewis *et al.*, 1985),

and C.A. Horn (unpublished results) has found a transient LH pulse on this day. In the opossum, peak values of oestradiol are detected immediately and up to 4 days before oestrus (Harder and Fleming, 1981) (Figure 7.5). However, in the kowari *Dasyuroides byrnei*, ovulation occurs 4–6 days post-oestrus but there is a pulse of LH 12 days before oestrus (Fletcher, 1983).

Ovulation, which, as far as is known, is spontaneous in all marsupials, occurs in most marsupials 1–2 days after oestrus, though it occurs up to 10 days later in three dasyurids (Hill and O'Donoghue, 1913; Woolley, 1966; Selwood, 1980; Fletcher, 1983). Marsupial oocytes have no surrounding cumulus or corona cells but are covered by a thin zona pellucida of about 4 μm in the opossum *Didelphis virginiana* (Rodger and Bedford, 1982a) and 3 μm in the dunnart *Sminthopsis* (Breed and Leigh, 1988) (Figure 7.6).

Mucoid material is deposited around the zona soon after the egg enters the oviduct. This mucopolysaccharide layer, which increases in thickness as the egg moves down the oviduct, acts as a secondary block to

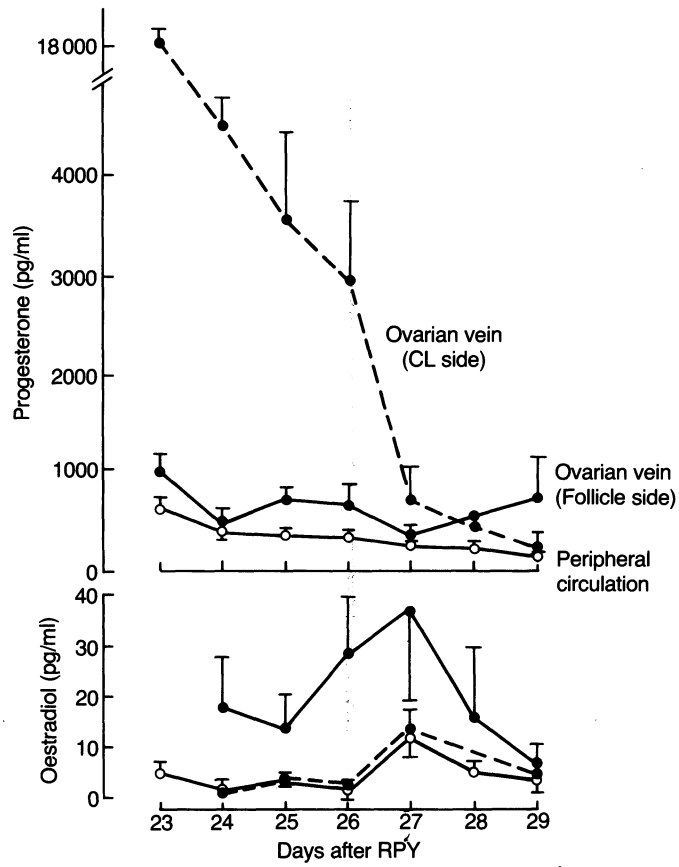


Figure 7.4 Concentrations (mean ± SEM) of progesterone and oestradiol-17β in the peripheral circulation (○ — ○), the ovarian vein draining the ovary with the corpus luteum (● - - - ●) and the ovarian vein draining the ovary bearing the Graafian follicle (● _ ●) through late pregnancy, parturition, and postpartum oestrus in the tammar wallaby. Data for oestradiol from Harder *et al.*, 1984. (Redrawn from Tyndale-Biscoe *et al.*, 1986.)

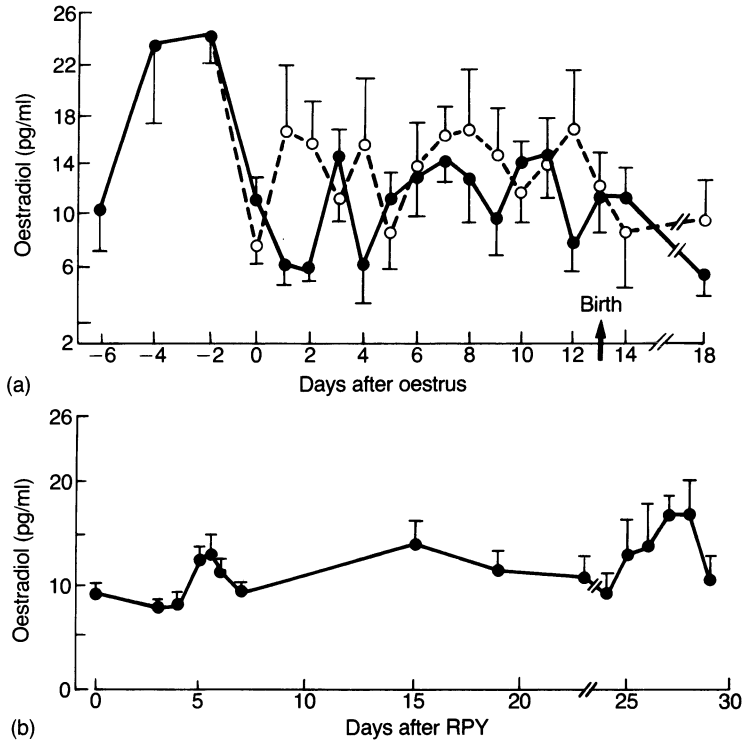


Figure 7.5 (a) Concentrations (mean ± SEM) of oestradiol and progesterone in the peripheral circulation of *Didelphis virginiana* during the oestrous cycle (● — ●) and gestation (○ — ○). Data from Harder and Fleming (1981). (b) Concentrations (mean ± SEM) of plasma oestradiol in *Macropus eugenii* between days 0 and 30 after removing pouch young. Note that, after day 24, the values are synchronized to the time of birth rather than to day after RPY. There are two significant rises in oestradiol: at day 5, coincident with the progesterone peak, and one day after birth, coincident with oestrus and mating. Data from Shaw and Renfree, 1984. (From Tyndale-Biscoe and Renfree, 1987, with permission).

polyspermy (Rodger and Bedford, 1982a; Selwood, 1982; Breed and Leigh, 1988; Breed *et al.*, 1989). The primary block to polyspermy may be due to a release of cortical granules beneath the vitelline membrane, as found in eutherians and observed in *Didelphis* (Rodger and Bedford, 1982a) and in an unfertilized *Sminthopsis* egg (Breed and Leigh, 1988). Fertilized *Sminthopsis* eggs lack these cortical granules but the block to polyspermy appears to be inefficient (Breed and Leigh, 1990). Fertilization can therefore only be effected by spermatozoa which have already reached the fimbria (Rodger and Bedford, 1982b; Rodger, 1990), and in the tammar they are at this location 24 h post coitum (p.c.) (Tyndale-Biscoe and Rodger, 1978). Oestrus must therefore precede ovulation by at least this time if fertilization is to occur before the mucin layer is too thick. Transport down the oviduct is also rapid, and all marsupial fertilized eggs are transported to the uterus in less than a day, while the keratinous shell membrane secreted by cells in the lower oviduct and utero-tubal region is laid down over the mucoid coat (Tyndale-Biscoe and Renfree, 1987). The first cleavage division usually takes place within the uterus between 30 and 40 h post-ovulation (Figures 7.6 and 7.7), and the six or seven cleavage divisions which lead to the blastocyst stage are complete by day 4 in the opossum *Didelphis*, day 5

in the brush-tailed possum *Trichosurus*, day 8 in the brown antechinus *Antechinus stuartii* and quoll *Dasyurus viverrinus* and day 8–9 in the tammar (Tyndale-Biscoe and Renfree, 1987).

In the common dunnart, *Sminthopsis macroura*, the time taken to reach the expanding complete unilaminar blastocyst of about 32 cells is just less than 4 days (Selwood, 1987). In this and in the fat-tailed dunnart, *S. crassicaudata*, there is an arrest of 24 h at the four cell stage, while in *Antechinus stuartii* cleavage is arrested for 4 days at the four cell stage (Selwood, 1980, 1981, 1986,

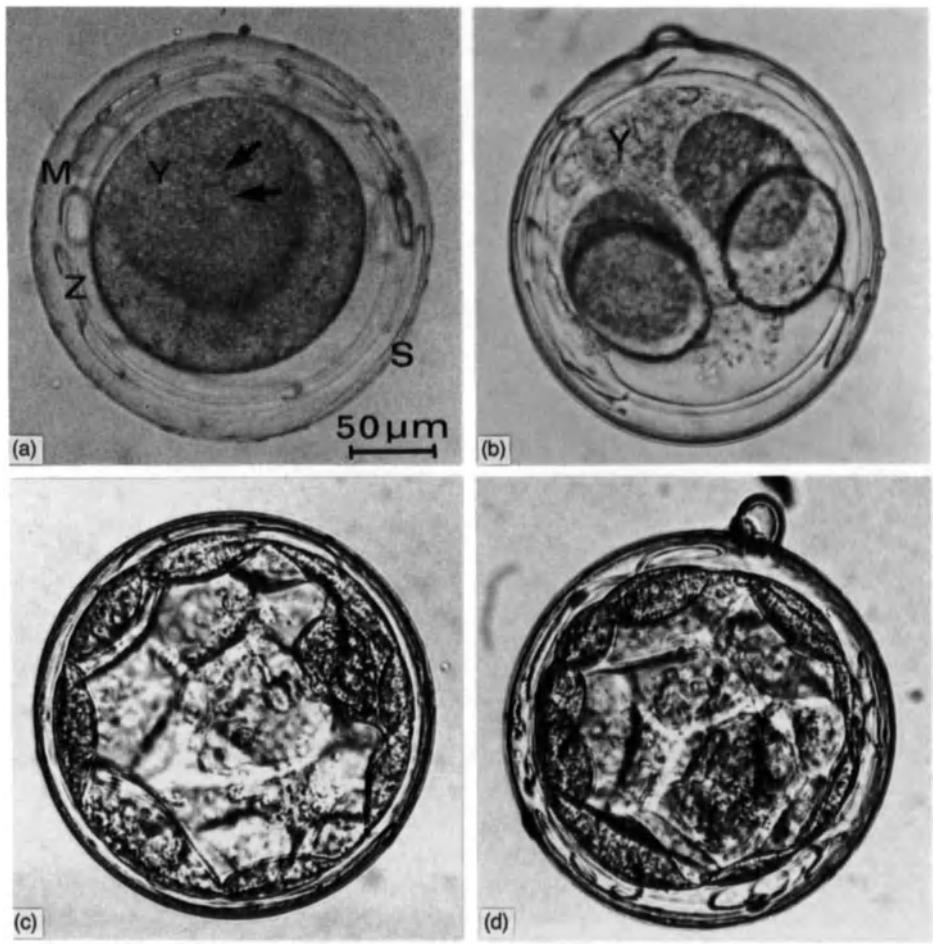


Figure 7.6 Cleavage stages and blastocyst formation in a dasyurid, *Sminthopsis crassicaudata*.
(a) The fertilized egg. The two pronuclei (arrows) can be seen through the yolk mass (Y) that has started to accumulate at one pole of the vitellus. Many sperm tails are visible in the mucoid layer (M). S, shell; Z, zona pellucida. (b) An embryo with four rounded blastomeres. The yolk mass (Y) lies at one pole of the embryo. An imperfection in shell deposition is found at this pole, which lies at the top of the figure. (c) An embryo at the 16-cell stage viewed from the non-yolky pole. The roof of the blastocyst is completely lined by cells over the transparent yolk mass, which is not in focus in the blastocoele. (d) An incomplete blastocyst of about 16 cells. The blastocyst is viewed from the open non-yolky hemisphere looking into the blastocoele. An imperfection in the shell deposition lies to one side of the embryo. (Photographs kindly provided by Dr Lynne Selwood.)

1987; Selwood and Young, 1983; Selwood and Santhananthan, 1988). Whilst these first cleavage events are occurring, there are no remarkable changes in the circulating steroids in the tammar, opossum, brush possum, brown antechinus or quoll (Harder and Fleming, 1981; Hinds and Tyndale-Biscoe, 1982a; Curlewis *et al.*, 1985; Hinds, 1989a; Hinds and Selwood, 1990), and indeed in *S. macroura* and *S. crassicaudata* addition of progesterone to the medium does not improve culture success of cleavage-stage eggs. As in eutherian mammals, the cleaving egg appears to be autonomous, and this is supported by evidence of blastocyst formation in quokkas, tammars and brush possums ovariectomized at the time of the first cleavage (Tyndale-Biscoe, 1963a, 1970; Sharman and Berger, 1969; Shorey and Hughes, 1973a), and by *in vitro* culture studies (Selwood, 1987).

In the majority of marsupials the concentrations of progesterone steadily increase during early pregnancy and, as noted above,

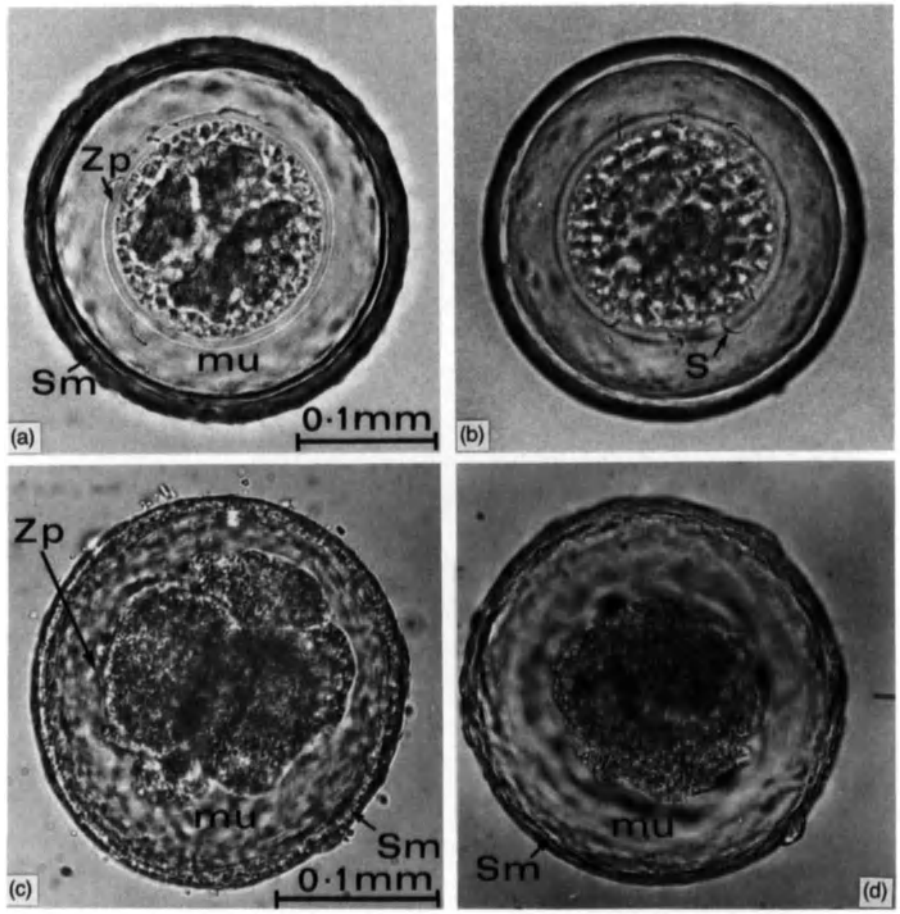


Figure 7.7 Early cleavage stages in an acrobatid, the feather-tailed glider, *Acrobates pygmaeus* (a and b), and a macropodid, the tammar wallaby, *Macropus eugenii* (c and d). (a, b and c) Four-cell embryos; (d) 20-cell embryo. Sm, shell membrane; mu, mucoid coat with embedded spermatozoa (s); and Zp, zona pellucida. In both species there is a considerably thicker mucoid layer than in the dasyurids (see Figure 7.6), and much less yolk. a and b to same scale; c and d to same scale.

formation of the blastocyst does not apparently require any specific stimulus. However, continued development and expansion of the blastocyst requires the continued secretion of progesterone at least to day 10 in the brush possum (Sharman, 1965a) and the opossum (Renfree, 1974), but only to day 8 in the quokka and tammar (Tyndale-Biscoe, 1963a, 1970). In these last two (macropodid) species, it is significant that this is after an early, transient pulse of progesterone which occurs at day 3–4 in the quokka and day 5–7 in the tammar (Cake *et al.*, 1980; Hinds and Tyndale-Biscoe, 1982a). This period also coincides with increased growth of the endometrium and increased uterine secretions, and will be discussed in detail in the section 'Maintenance of pregnancy'. In the absence of this transient pulse of progesterone, embryos at the blastocyst stage enter embryonic diapause.

7.4 EMBRYONIC DIAPAUSE

Blastocyst growth in mammals that do not have embryonic diapause occurs by rapid cell division and fluid accumulation. Although diapausing blastocysts can also accumulate fluid, there appears to be little or no mitotic activity and therefore little or no new cell proliferation (Daniel, 1970; Renfree, 1978). In mammalian blastocysts dormancy occurs when the embryo is 200–400 cells with the exception of the badger (900–2000), macropodid marsupials and rodents (80–100 cells) and tarsipedid, burramyid and acrobatid marsupials, in which the blastocysts are up to 2000 cells (Renfree, 1981a; S. Ward and Renfree, 1988; S. Ward, 1988). With the one exception of *M. fuliginosus*, all species of the two subfamilies of the Macropodidae that have been investigated exhibit the phenomenon of diapause (Tyndale-Biscoe *et al.*, 1974; Renfree, 1981a). During diapause the blastocyst size (shell diameter) varies from 0.25 to 0.33 mm (M.J. Smith, 1981) and the diameter of the trophoblast of four macropodid species varies little – from 0.20 to 0.25 mm (Tyndale-Biscoe, 1963b; Renfree and Tyndale-Biscoe, 1973; Tyndale-Biscoe and Renfree, 1987). The macropodid blastocyst in diapause is invariably unilaminar and consists of 70–100 uniform cells in which mitoses are never seen (Figures 7.8 and 7.9). Blastocysts in diapause are always associated with a quiescent corpus luteum.

The Macropodidae are monovular and polyoestrous marsupials, and gestation is longer than the oestrous cycle in all except *Wallabia bicolor*, the swamp wallaby (Tyndale-Biscoe and Renfree, 1987). Some species have long oestrous cycles, so that birth occurs before the pro-oestrous phase is initiated, and sucking prevents further development (see Figure 7.2). In these species (*M. giganteus, M. parma, M. parryi* and *Aepyprymnus rufescens*), diapause usually occurs after fertilization in late stages of pouch life (Calaby and Poole, 1971; Maynes, 1973; Tyndale-Biscoe, 1973; Moors, 1974; Poole and Catling, 1974; Tyndale-Biscoe *et al.*, 1974). *M. fuliginosus*, as mentioned above, does not ever have diapause since the lactational inhibition of ovarian activity is complete and the pro-oestrous phase of the cycle is suppressed throughout pouch life. If the suppression fails, as it occasionally does, the resulting conception can occur during lactation but there is no delay and the neonate is born into a pouch still occupied by a larger young and it dies within a few days (Poole, 1975). This supports Sharman's (1965a) idea that the prime importance of diapause in macropodids is to prevent occupation of the pouch by two young of different ages, an event that would occur regularly since oestrus and ovulation are not normally inhibited postpartum. This is equivalent to the prevention of superfetation by ovulation inhibition during pregnancy in eutherians.

In the remaining macropodids, gestation extends into the post-luteal and pro-oestrous phases without suppressing follicle growth, so that ovulation occurs a few days after par-

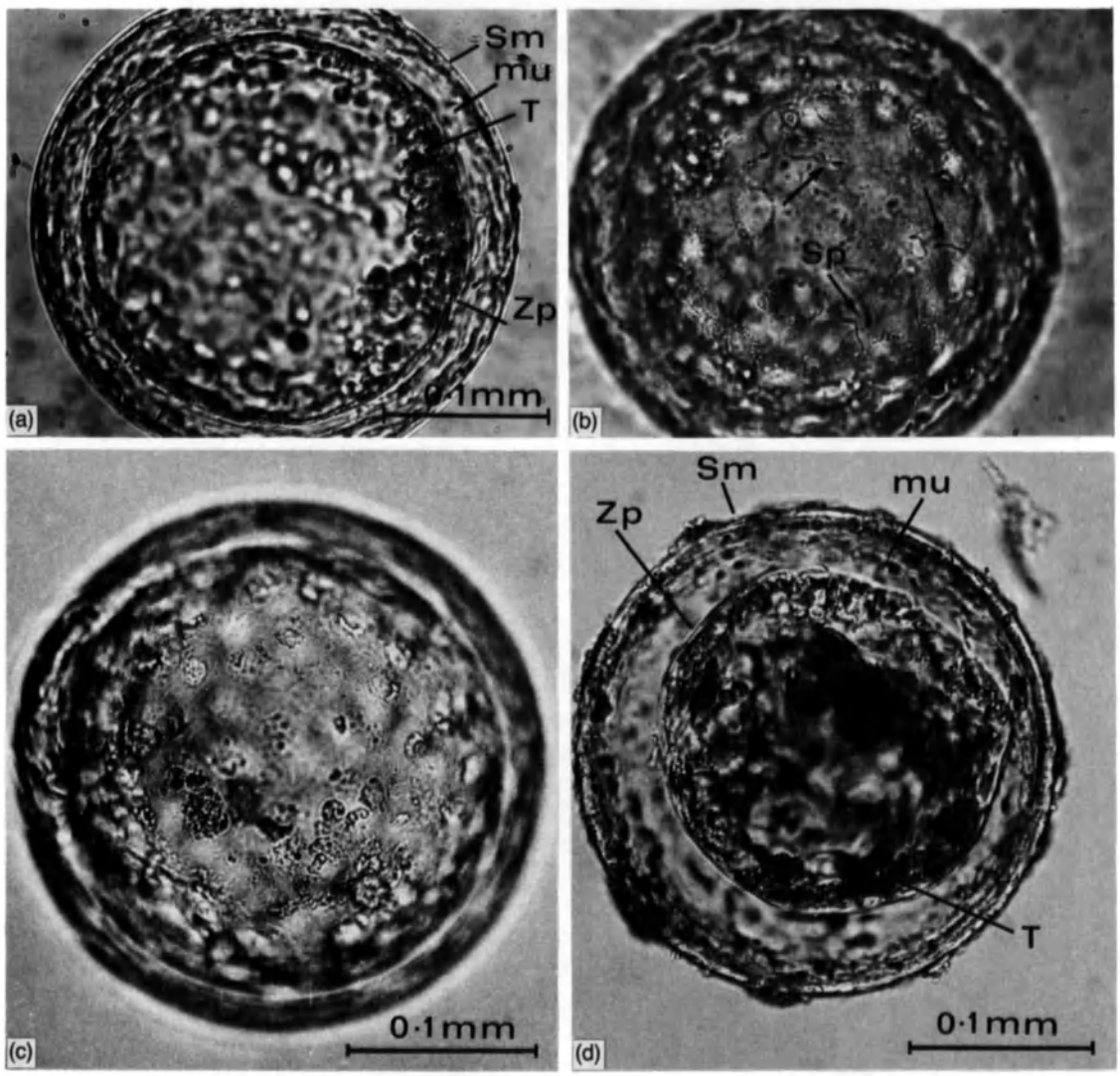
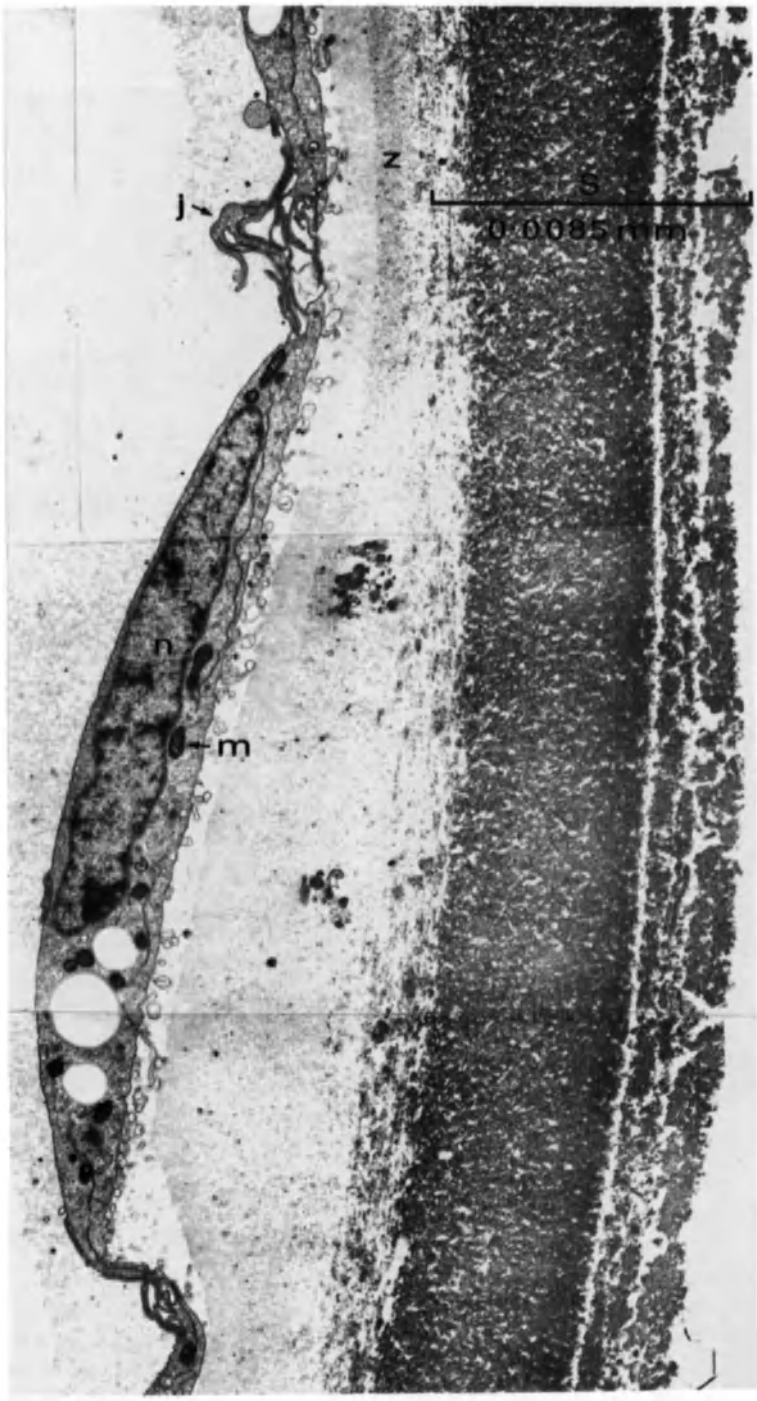


Figure 7.8 Living macropodid marsupial blastocysts in embryonic diapause. (a and b) Tammar, *Macropus eugenii*; (c) Potoroo, *Potorous tridactylus*; and (d) Quokka, *Setonix brachyurus*. (a) A mid-focused photograph shows the unilaminar layer of trophoblast (T) cells, surrounded by a thin zona pellucida (Zp), a thick mucin layer (mu) and shell membrane (Sm). (b) A surface focus of the same embryo at the same magnification shows the numerous spermatozoa trapped in the mucoid coat. (c) and (d) are focused at a similar level to (a). Sm, shell membrane; Sp, spermatozoa; mu, mucoid layer; Zp, zona pellucida; T, trophoblast. (c courtesy of Dr G. Shaw; d courtesy of G.I. Wallace.)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turition in these species (e.g. *Setonix brachyurus, Macropus eugenii, Macropus rufogriseus, Megaleia rufa, Bettongia lesueur, Thylogale billardierii,* and *Potorous tridactylus*; Tyndale-Biscoe and Renfree, 1987). Sucking prevents the full development of the corpus luteum, and if fertilization has occurred post-partum the embryo is retained as a unilaminar blastocyst in diapause (Sharman, 1955a,b; Hughes, 1962; Sharman, 1965b; Tyndale-Biscoe, 1963b, 1968; Calaby and Poole, 1971; Rose and McCartney, 1982; Figures 7.8 and 7.9).

In addition to the lactational inhibition of the blastocyst and corpus luteum, in at least two species, the tammar *Macropus eugenii* and the Bennett wallaby *Macropus r. rufogriseus*, the quiescent blastocyst and corpus luteum persist for several months after weaning (Sharman and Berger, 1969), and resume development after the summer solstice (Berger, 1966; Renfree and Tyndale-Biscoe, 1973; Tyndale-Biscoe *et al.*, 1974; Merchant and Calaby, 1981; Tyndale-Biscoe and Renfree, 1987).

Until recently, amongst marsupials diapause was thought to be restricted to the macropodids, but Tyndale-Biscoe (1973) was the first to suggest that Hill's (1900) observations on *Acrobates* were consistent with the occurrence of diapause. Confirmation has now been provided for three non-macropodid families: the Tarsipedidae, a monospecific family consisting only of *Tarsipes rostratus* (the honey possum) (Renfree, 1980a, 1981a; Renfree and Calaby, 1981; Wooller *et al.*, 1981); Burramyidae (the pygmy possums), *Cercartetus concinnus* (Clark, 1967); *Cercartetus nanus* and *C. lepidus* (Ward, 1988), and Acrobatidae, *Acrobates pygmeaus* (the feather-tailed glider) and *Distoechurus pennatus* (the feather-tailed possum) (S. Ward and Renfree, 1988; S.Ward, 1988). Graafian follicles are found in the ovaries of females with newborn young, and sperm from a post-partum oestrus mating are found in the reproductive tract (Figure 7.10).

In these small possums the diapause blastocyst is very large (1–2 mm) and consists of about 2000 cells in *Acrobates* and *Tarsipes* (Figure 7.11).

In contrast to the macropodids, there is a period of slow growth (for about 30 days) of both the blastocyst and corpus luteum early in the diapause (Renfree, 1981a; S. Ward and Renfree, 1988) (Figure 7.12).

Nothing is yet known of the endocrine control of diapause in these small possums but it does not appear to be under lactational control (Renfree *et al.*, 1984a; S. Ward and Renfree, 1988). It may be related to the birth of the previous offspring or to nutritional or ecological factors. Since these species have a seasonal pattern of reproduction, S.Ward (1988) has suggested that nutrition and availability of nectar and pollen-bearing flowers may be an important controlling factor.

7.5 INITIATION AND MAINTENANCE OF DIAPAUSE

Blastocysts in diapause are always associated with a quiescent corpus luteum, and in the quokka the blastocyst degenerates when the corpus luteum disappears from the ovary (Sharman and Berger, 1969) and when the peripheral plasma progesterone levels are

Figure 7.9 Transmission electron micrograph of one cell of a 100-cell quiescent tammar blastocyst taken from the uterus of a lactating female. The shell membrane has the characteristic three layers, with the mucin coat separating it from the zona pellucida. The individual trophoblast cells are flattened along the inner surface of the shell, with elongate nucleii. Convoluted tight junctions join adjacent trophoblast cells. No sperm fragments are seen in this micrograph (×5500). The scale bar shows the width of the shell membrane as 0.0085 mm. j, junctional complex; m, mitochondrion; n, nucleus; S, shell membrane; z, zona pellucida.

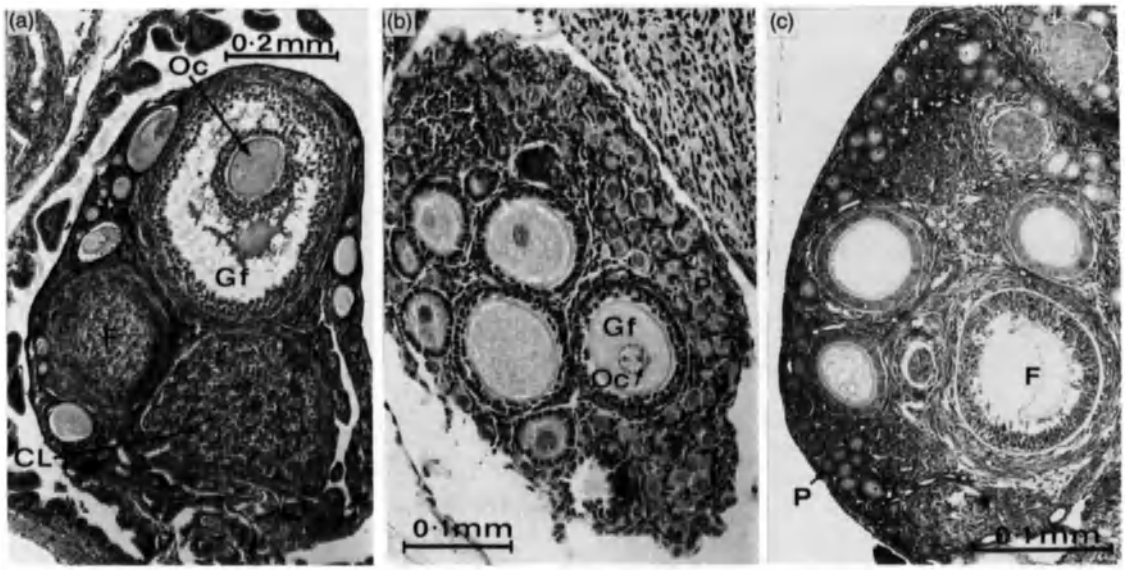


Figure 7.10 Folliculogenesis in the ovaries of three small possums. (a) The feather-tailed glider, *Acrobates pygmaeus*. Section of the whole ovary of a female with a large Graafian follicle (Gf) and enclosed oocyte (Oc), together with a surface section of another follicle (F), numerous primary follicles and a mature corpus luteum (CL). Note the absence of obvious regions of interstitial tissue. (b) The honey possum, *Tarsipes rostratus*. Section of the whole ovary of a female with a follicular oocyte (Oc). Three Graafian follicles (Gf) and three secondary follicles are evident, but there appears to be relatively little discrete interstitial tissue. No corpora lutea are visible in this section. (c) The monitos del monte, *Dromiciops australis*. Section of the ovary showing several developing follicles and numerous primary follicles (P) in the cortex. No corpora lutea are visible and, as in (a) and (b), no discrete interstitial tissue is present. [c kindly provided by Dr P. Temple-Smith and the Corporacion Nacional Forestal (CONAF) Chilé, which gave him permission to collect *Dromiciops* specimens.]

very low (G.I. Wallace, personal communication). The corpus luteum, however, is not required to maintain diapause because blastocysts can remain apparently viable for at least 4 months after ovariectomy or luteotomy (Tyndale-Biscoe and Hearn, 1981). In the brush-tailed bettong it appears that the corpus luteum may have an inherent lifespan of about 80 days and is not influenced by either sucking or season (M.J. Smith, 1989).

As noted in the previous section, the corpus luteum is not necessary for entry into diapause, and its removal at day 2 p.c. allows the one-cell fertilized uterine egg to progress to the blastocyst stage (Sharman and Berger, 1969). Tyndale-Biscoe (1979) has shown that the 6–7 cell divisions required to reach the unilaminar blastocyst stage of 80–90 cells takes 6–8 days from fertilization, which is about 7 days from oestrus. This is twice as long as cleavage and blastocyst formation in the opossum and bandicoot (Hartman, 1928; Lyne and Hollis, 1976). Expansion of tammar blastocysts in non-lactating females has occurred by day 9 (Figure 7.13), and endometrial weights have also increased by this time, with a distinct change in endometrial proteins occurring at day 8 p.c. (Tyndale-Biscoe, 1979, 1986).

Thus the inhibitory influence of the sucking stimulus begins to take effect before day 8 p.c. This has been further investigated by removal of the sucking young at daily intervals from birth, and measuring the interval to the next birth (Tyndale-Biscoe, 1986). In non-lactating females, in which there is no dia-

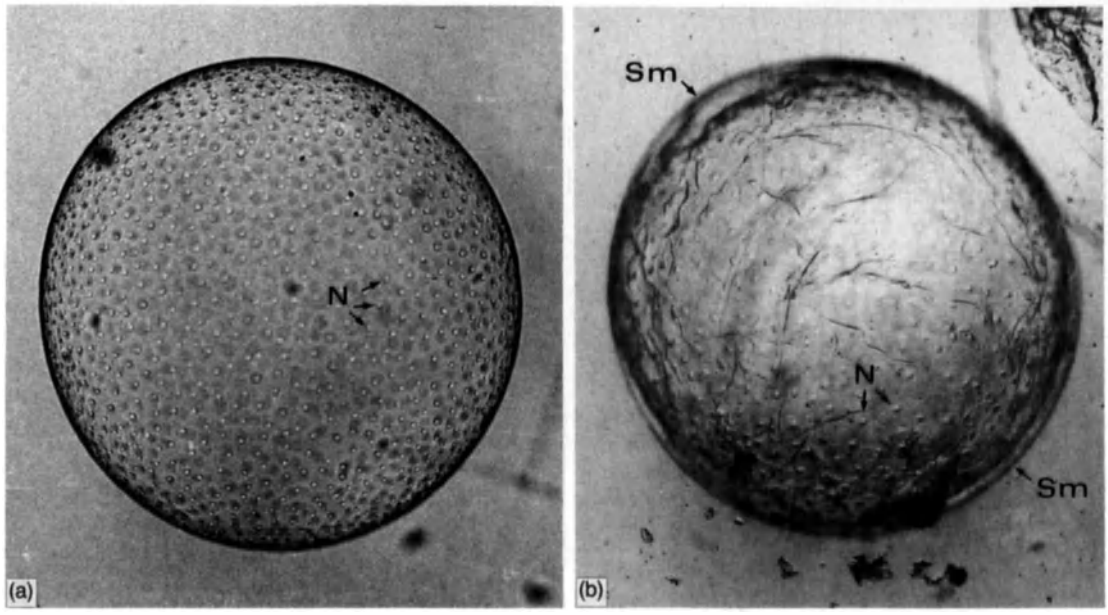


Figure 7.11 Diapause. Blastocysts of two small possums, the feather-tailed glider, *A. pygmaeus*, and the honey possum, *T. rostratus*. (a) Living 1.7-mm-diameter blastocyst from uterus of a lactating female *A. pygmaeus*, containing approximately 2000 cells. N, trophoblast nuclei. (b) Living 1.2-mm-diameter blastocyst from the uterus of a lactating female, *T. rostratus*. There can be variation in size between the 2–3 blastocysts in each uterus, but most have reached this size by day 30 of pouch life and undergo little further expansion until the end of lactation (see Figure 7.12). Sm, shell.

pause, the interval from oestrus to birth is 29 or 30 days, but the interval from RPY to birth remains constant at 26.4 days on day 4 or 5 after oestrus. The sucking inhibition thus begins on day 4 or 5 post oestrus. In the absence of any sucking stimulus, there is a transient pulse of progesterone on days 6 or 7 post oestrus (Hinds and Tyndale-Biscoe, 1982a). Two days after the progesterone pulse there is an increase in the weight of the endometrium and a change in the composition of the uterine secretions, and expansion of the blastocyst. The interval from the progesterone pulse to birth is relatively constant at 21–22 days, regardless of whether or not the female is lactating (Tyndale-Biscoe and Renfree, 1987) (see Figure 7.14).

One interesting variant on this general control mechanism for the entry into diapause occurs in the pubertal tammar (and, presumably, Bennett's) wallabies in which seasonal, photoperiodic influences can act directly without the presence of any sucking stimulus. Young females leaving the pouch in October and early November during seasonal quiescence may ovulate, mate and develop a conceptus to the 100-cell blastocyst stage (Andrewartha and Barker, 1969; Tyndale-Biscoe and Hawkins, 1977). This blastocyst then enters embryonic diapause and it remains quiescent until the subsequent summer solstice in late December (Berger and Sharman, 1969a).

7.6 REACTIVATION AFTER DIAPAUSE

7.6.1 THE ROLE OF THE SUCKING STIMULUS

It is clear from the studies summarized above that the influence of the sucking stimulus is all-important in the maintenance of lactational quiescence in all but pubertal animals, at

least amongst macropodids (Tyndale-Biscoe and Renfree, 1987). Not surprisingly, it is the neural stimuli from the teat that is of primary importance, and denervation of the mammary gland in the tammar causes immediate reactivation of the diapause blastocyst even though lactation continues (Renfree, 1979). However, development does not resume immediately on removal of the sucking stimulus, in that no changes can be seen in the blastocyst before day 8, and no change in the corpus luteum before day 4. Birth does not occur 21–22 days after RPY, which is the time taken from the early progesterone pulse in non-lactating females to birth. The actual time taken is around 5 days longer than this.

After removal of pouch young, there is a critical period after day 2 and before day 4 when ovariectomy or excision of the corpus luteum is followed by a resumption of blastocyst growth, but subsequent collapse, and by a failure of the endometrium to become secretory (Berger and Sharman, 1969b). Ovariectomy or lutectomy before this stage blocks reactivation, and after this stage allows fetal development to full term (Berger, 1970; Tyndale-Biscoe, 1970; I.R. Young and Renfree, 1979). Similarly, lutectomy at day 3 RPY results in a failure to produce a neonate but a new ovulation occurs instead 18–23 days after RPY (Fletcher and Renfree, 1988). When reactivation is stimulated by exogenous progesterone, which by-passes the corpus luteum, the fetus takes 3 days less to reach full term than after RPY (Figure 7.14) so it has been assumed that the corpus luteum takes 3 days to reactivate after sucking stimulus withdrawal (Renfree and Tyndale-Biscoe, 1973; Tyndale-Biscoe, 1979). Recently we have investigated the precise time for which

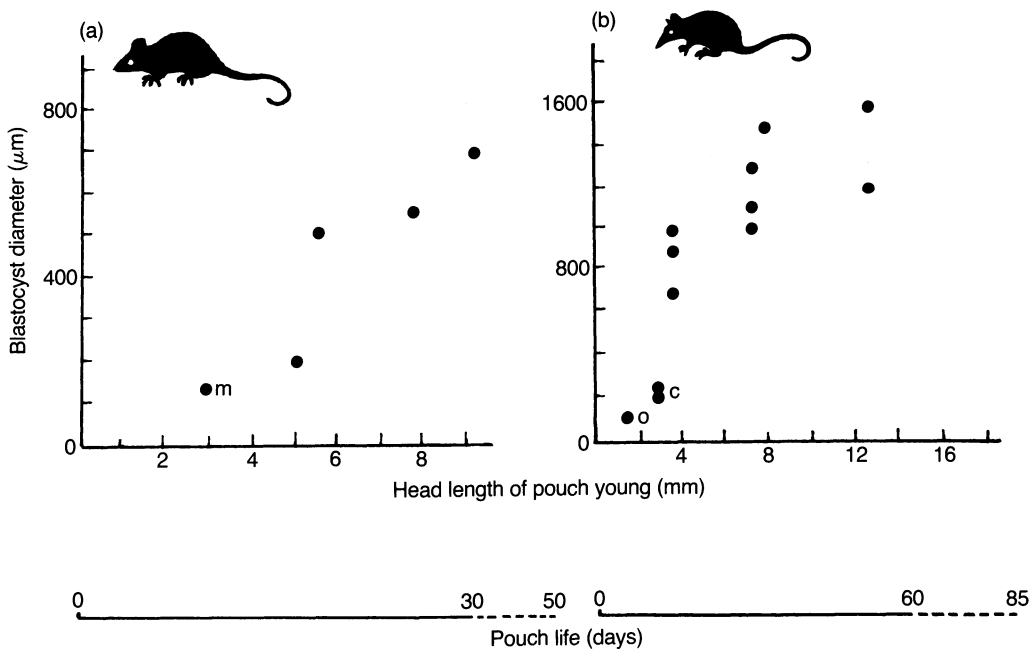


Figure 7.12 Blastocyst growth during lactation in (a) the pygmy possum, *Cercartetus concinnus*, and (b) the honey possum, *Tarsipes rostratus*, shown in relation to size of young in the pouch. Based on data from Bowley (1939), Clark (1967) and Renfree (1980a). The duration of pouch life (solid line) and suckling after pouch exit (broken line) are indicated below: o, oocyte in follicle; c, cleaving eggs; m = mitotic figures observed. (From Tyndale-Biscoe and Renfree, 1987, with permission.)

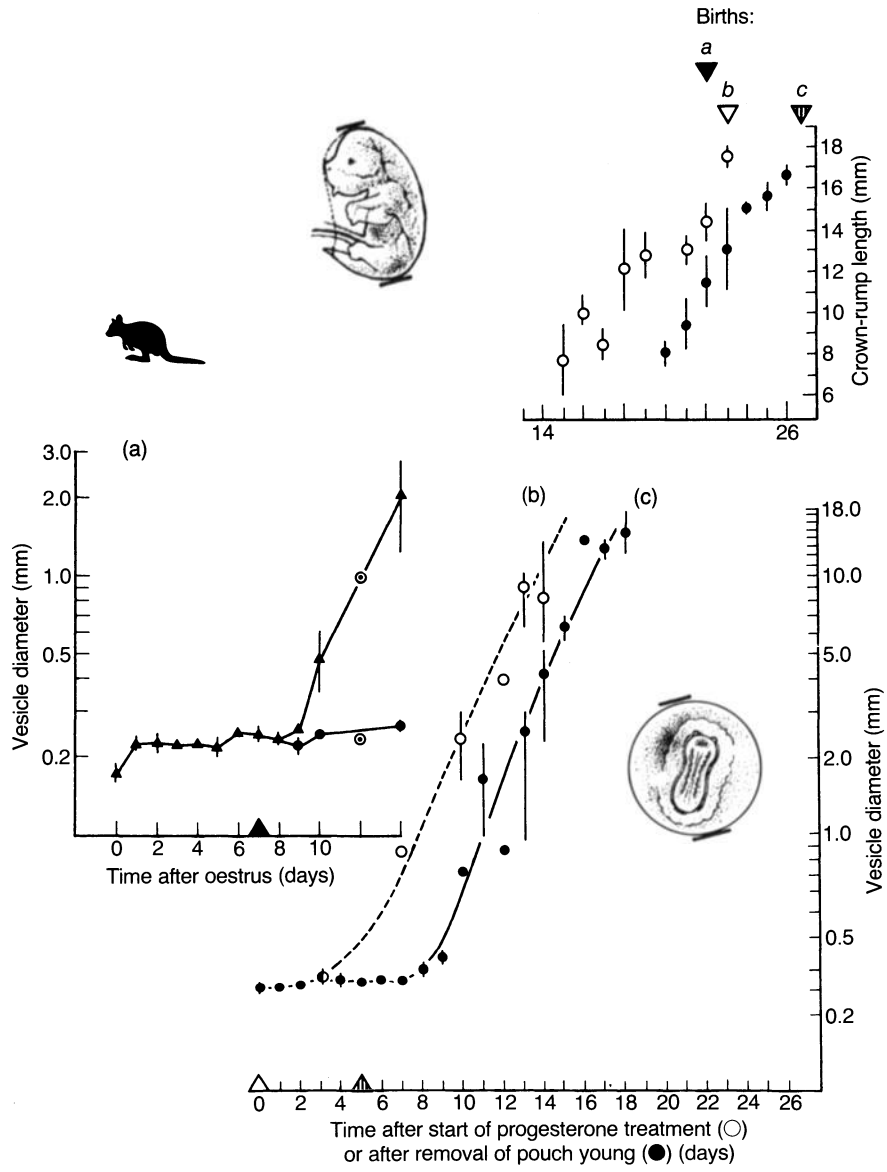


Figure 7.13 Growth of the embryo of *M. eugenii* before and after lactation. (a) Growth of the conceptus after oestrus uninhibited by the presence of (▲) a sucking pouch young compared with (•) lack of growth in the presence of a neonatal young. ▲ represents the mean day of transient pulse of progesterone in non-lactating animals; •, mean values for pregnant animals ovariectomized (lower point) or sham operated (higher point) on day 2 by Sharman and Berger (1969). (b) Growth of the diapausing blastocyst stimulated by 10 days of progesterone injection (○) or (c) after RPY (•). Δ, start of progesterone treatment: ⩓ , mean day of transient pulse of progesterone after RPY. Time of birth of treated animals shown in top panel a: ▼ (day 29 after oestrus), b: ▽ (day 23 after start of progesterone treatment) and c: ⩛ , (day 27 after RPY). Note the 3–4 day advance in time of birth after progesterone treatment compared with RPY. (From Tyndale-Biscoe and Renfree, 1987, with permission.)

the sucking stimulus must be withdrawn to reactivate the corpus luteum and, if present, the diapausing blastocyst, and have found once again that 3 days is required. The sucking stimulus must be absent for at least 72 h to allow the corpus luteum to escape inhibition (Gordon *et al.*, 1988). If the young is returned to the teat and reattaches after 72 h, reactivation is not inhibited and pregnancy proceeds concurrent with lactation. Collectively the evidence demonstrates that the corpus luteum must be present for 3 days if reactivation is to be initiated (see Figure 7.19), presumably to mediate the effects of the loss

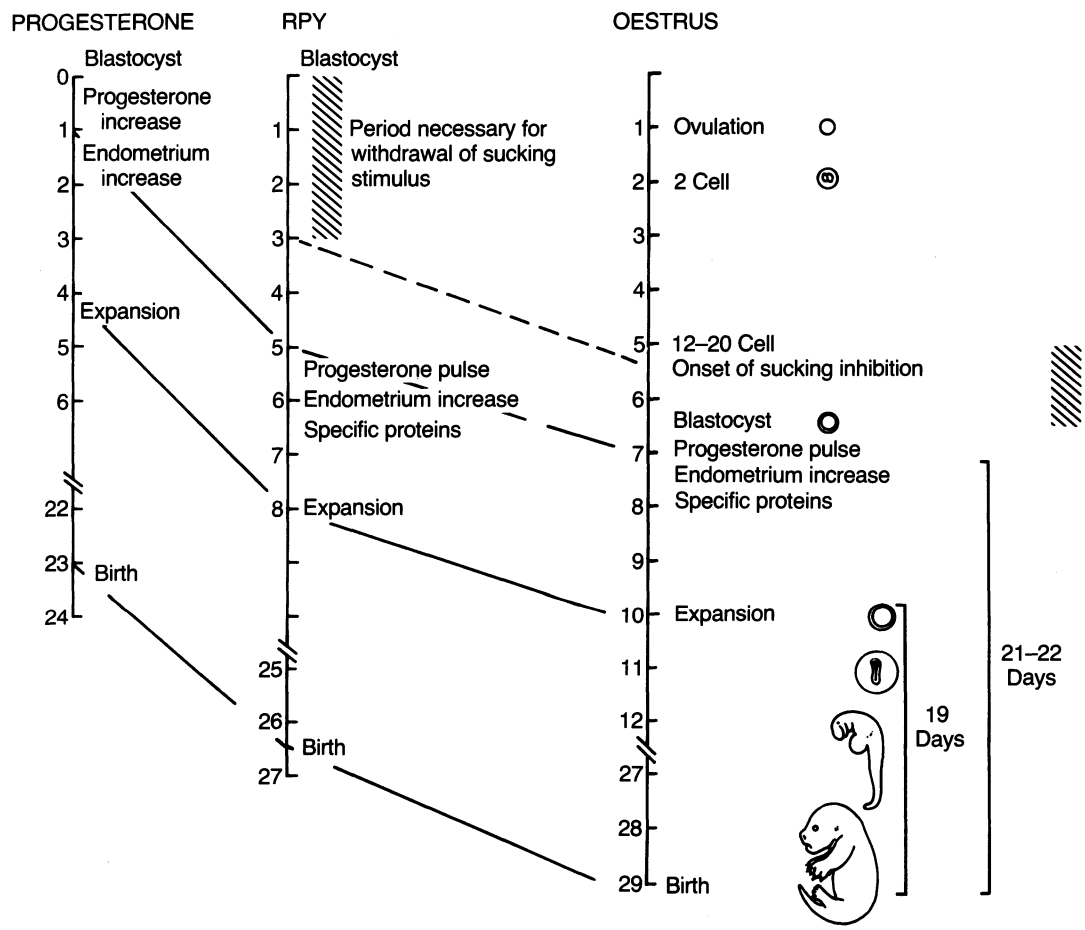


Figure 7.14 Intervals from either exogenous progesterone injection, removal of pouch young or oestrus to the withdrawal or onset of sucking inhibition, the early rise in progesterone (progesterone pulse), the increase in endometrial wet weight, the first measurable expansion of the blastocyst and birth. Although not shown, the progesterone pulse also coincides with an oestradiol-17β pulse after RPY. Note that, whilst the timing from injection, RPY or oestrus to the early progesterone pulse or to expansion is variable, the duration of pregnancy from the pulse is more or less constant and takes 21–22 days (Tyndale-Biscoe & Renfree 1987), and from expansion to birth 19 days. Compare also with Figure 7.19. Based on data in Renfree (1973a,b); Renfree and Tyndale-Biscoe (1973); Tyndale-Biscoe (1979, 1986); Hinds and Tyndale-Biscoe (1982a); Shaw and Renfree (1984); Fletcher and Renfree (1988); Gordon *et al.* (1988).

of the sucking stimulus. It also implies that nothing irreversible happens on day 1 and day 2 after RPY, but that the pituitary – hypothalamic axis must be sensitive to the lack of the sucking inhibition on three successive days. This will be discussed further below.

7.6.2 THE PITUITARY AND REACTIVATION

The marsupial corpus luteum does not depend on a luteotrophic stimulus for reactivation and subsequent progesterone secretion, but rather needs the withdrawal of an inhibitory effect. In the tammar, the quiescent corpus luteum undergoes its normal growth and secretory functions after total hypophysectomy (Hearn, 1973, 1974, 1975) with a transient pulse of progesterone 7 days after the operation (Tyndale-Biscoe *et al.*, 1986; Figure 7.15).

Hearn's (1974) results did not differentiate between the anterior and posterior pituitary. Sharman (1965a) showed that oxytocin injection (and water!) could prolong quiescence, and Tyndale-Biscoe and Hawkins (1977) showed that both oxytocin and prolactin delay reactivation (see Figure 7.16).

However, after hypophysectomy only prolactin has this effect, and it is now generally accepted that during diapause prolactin is the inhibitory agent and administration of prolactin after removal of the pouch young prevents corpus luteum growth (Figure 7.16) (Tyndale-Biscoe and Hawkins, 1977; Hinds, 1989b). Confirmation of the importance of prolactin in this process is provided by the finding that the corpus luteum contains prolactin (Sernia and Tyndale-Biscoe, 1979) but not LH receptors (F.Stewart and Tyndale-Biscoe, 1982; Figure 7.17).

However, neither prolactin nor LH has any effect on steroidogenesis by luteal tissue *in vitro*, so that once the inhibition is removed the corpus luteum resumes its autonomous life (Sernia *et al.*, 1980; Hinds *et al.*, 1983; Tyndale-Biscoe, 1986). Removal or replacement of pouch young in early lactation does not cause a marked change in prolactin concentrations (Hinds and Tyndale-Biscoe, 1982b; Gordon *et al.*, 1988), and although a single injection of the dopamine agonist bromocriptine initiates blastocyst reactivation (Table 7.2) there is likewise no change in prolactin concentration measured in once-daily samples (Tyndale-Biscoe and Hinds, 1984). Although it remains possible that a prolactin pulse may have been abolished but not detected by this sampling regimen (Hinds and Tyndale-Biscoe, 1985; Tyndale-Biscoe *et al.*, 1986; Gordon *et al.*, 1988), the time from RPY to birth is the same as bromocriptine injection to birth and the time from

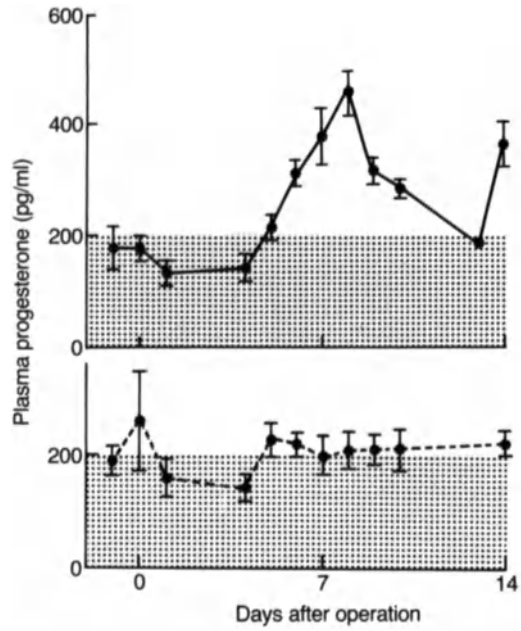


Figure 7.15 Responses in the concentrations (mean ± SEM) of progesterone in lactating tammars subjected to hypophysectomy (n = 4) (solid line) or sham hypophysectomy (n = 5) (dotted line). Removal of the pituitary released the corpora lutea from inhibition and they underwent normal development, including a change in secretion rate at day 7–8 (7.3 ± 0.2), whereas the sham operated females remained in lactational quiescence. Stippling indicates normal background plasma progesterone concentration during quiescence. (Redrawn from Tyndale-Biscoe *et al.*, 1986).

the progesterone pulse to birth is the same in both cases (Tyndale-Biscoe *et al.*, 1986). So how can this be resolved with the evidence that the sucking stimulus must be removed for 3 days? Evidence from experiments on seasonal reactivation provide clues to the mechanism. Animals induced to reactivate by experimental manipulation of photoperiod alter the time of secretion of melatonin even on the first night of the change, so that there is a synchronized melatonin release with the dark phase (McConnell *et al.*, 1986). In these animals, there is also a loss of an early-morning pulse of prolactin, and this is lost some time before day 5 after photoperiod change (McConnell *et al.*, 1986). If exogenous melatonin is administered 3 h before the dark phase in an inhibitory photoperiod of 15L:9D, thus effectively making it into a permissive period of 12L:12D, the prolactin pulse is abolished by day 3, but not by day 1, of this treatment (Tyndale-Biscoe *et al.*, 1986). The 3 day lag between removal of the sucking stimulus to irreversible reactivation must therefore be the time taken for the hypothalamic – pituitary axis to adjust to the loss of at least three consecutive dawn pulses of prolactin, presumably by withdrawal of prolactin release. However, bromocriptine does not apparently depress peripheral prolactin, although it may have abolished a dawn pulse (Tyndale-Biscoe *et al.*, 1986). Since Schallenberger *et al.* (1981) have demonstrated that a single injection of bromocriptine can suppress prolactin for 6 days in rhesus monkeys, it seems likely that it could act for at least the required 3 days in wallabies (Gordon *et al.*, 1988). This has recently been confirmed by monitoring the dawn pulse after photoperiod change and bromocriptine injection (Hinds, 1989b,c). The most critical factor for the maintenance of quiescence in the tammar is not sustained high concentrations of prolactin but the occurrence of a transient prolactin pulse. Daily injections of ovine prolactin maintained quiescence; endogenous prolactin induced by thyrotrophin-releasing hormone (TRH) also maintained quiescence, while half of the tammars treated with bromocriptine for 5 days lost their morning prolactin pulse and these reactivated. Similarly, the prolactin pulse is absent 5 days after a change to stimulatory photoperiods. Once the inhibitory effects are removed, by whatever means, the corpus luteum rapidly resumes progesterone production, which in turn induces a secretory uterus and blastocyst growth.

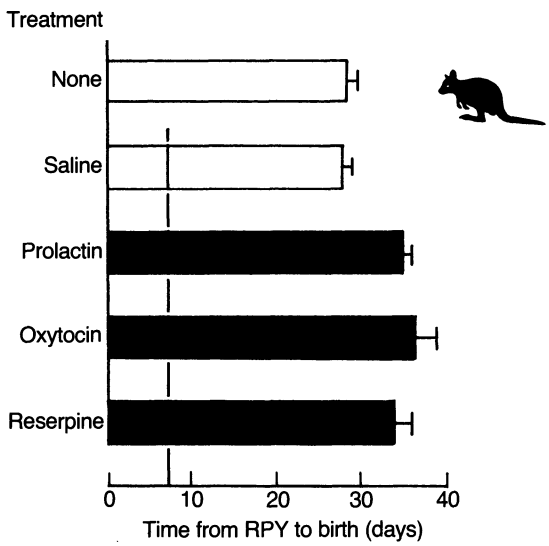


Figure 7.16 Time to birth or oestrus in *Macropus eugenii* from which the pouch young were removed (RPY) and the females variously injected three times daily for 7 days (vertical line). The apparent gestation period was extended by 7 days in the animals treated with oxytocin, prolactin and reserpine. Data from Tyndale Biscoe and Hawkins (1977). (From Tyndale-Biscoe and Renfree, 1987, with permission.)

7.6.3 REACTIVATION BEFORE THE PROGESTERONE PULSE

The first obvious change in peripheral plasma progesterone is the transient pulse on day 5 or 6, but there is evidence to suggest that both uterine and blastocyst reactivation have

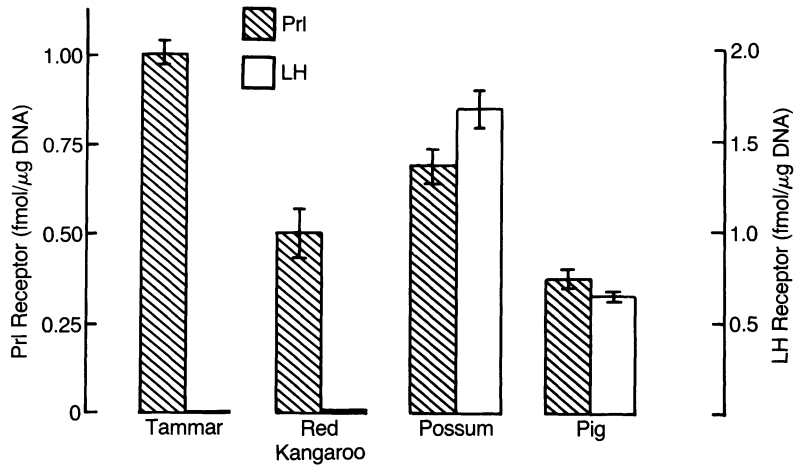


Figure 7.17 Prolactin (Prl) and luteinizing hormone (LH) receptors in luteal tissue of tammar, red kangaroo, brush-tailed possum and pig. Note that the corpora lutea of the two macropodid species contain no measurable LH receptors, but all four species have Prl receptors. Data from F. Stewart and Tyndale-Biscoe (1982).

Table 7.2 Response of tammars in lactational and seasonal quiescence to a single injection of bromocriptine (5 mg per kg body weight), at different months of the year. (From Tyndale-Biscoe *et al.*, 1986)

	Number treated	*Number reactivated*[a]	*Percentage reactivated*[a]
Lactational quiescence			
February	5	5	100
March	5	5	100
May	9	9	100
June	5	5	100
Seasonal quiescence			
June	20	20	100
July	47	38[b]	81
August 12	6	4[b]	67
August 26	6	6	100
September 9	6	2	33
September 22	16	0	0
October	5	0	0
November	5	1	20
December	5	0	0

[a] Reactivation determined by occurrence of progesterone pulse, birth, or oestrus.
[b] Reactivation determined only by occurrence of birth; others in these groups may have undergone a non-pregnant cycle that was not recorded.

already begun by this time. As noted above, between a critical period after day 2 and before day 6 after RPY in the quokka (Tyndale-Biscoe, 1963a) in the tammar (Sharman and Berger, 1969; Tyndale-Biscoe, 1970; I.R. Young and Renfree, 1979) and in the potoroo (Bryant and Rose, 1986) ovariectomy is followed by resumption of blastocyst growth and subsequent collapse and by failure of the luteal phase to develop in the endometrium. Ovariectomy after day 6 does not prevent the appearance of a luteal uterus and fetal development to full term. In both species initiation of reactivation precedes the time when an early pulse of progesterone occurs (Hinds and Tyndale-Biscoe, 1982a), and yet it appears that the corpus luteum is necessary to influence blastocyst reactivation, either directly or indirectly through increased or specific secretion from the uterus (see later sections for fuller discussion).

No gross changes can be observed in the blastocyst before day 8 (Renfree and Tyndale-Biscoe, 1973), but a few mitoses can be observed in the blastocyst on day 4 (Berger, 1970). The first significant increase in blastocyst diameter occurs by day 8, and a threefold increase by day 10 (Renfree and Tyndale-Biscoe, 1973). Between day 5 and day 10, the volume has increased 45-fold, and by day 15 by about 10 000-fold (Tyndale-Biscoe and Renfree, 1987). Endometrial protein synthesis increases as early as day 4, with a significant increase in leucine incorporation by the endometrium (Shaw and Renfree, 1986) and some prealbumins are present in uterine exudates at day 4 which are not present at day 0 (Renfree, 1973b). Metabolic reactivation of the blastocyst has occurred by day 5, as RNA polymerase activity and uridine incorporation are significantly higher in day 5 blastocysts than at day 4 and earlier stages (Moore, 1978; Thornber *et al.*, 1981; Shaw and Renfree, 1986). Glucose incorporation has also increased by day 5 RPY but is most significant between days 5 and 10 (Pike, 1981). These data suggest that a change in uterine secretion by day 4 causes embryonic reactivation by day 5 (Figure 7.18), which is also the day of the progesterone pulse and which also coincides with a pulse of oestradiol (Shaw and Renfree, 1984, 1986).

The conclusion must be that an earlier change is responsible for triggering reactivation of the blastocyst either directly or indirectly via the uterus. Most studies conclude that the blastocyst is indirectly stimulated by the progestational stimulus on the uterine secretions (Tyndale-Biscoe, 1970; Renfree, 1972a, 1973a), a conclusion supported by the recent results of Selwood (1987), who found that addition of progesterone to the culture medium did not improve the *in vitro* culture conditions for *Sminthopsis* cleaving and expanding blastocyst stages. Exogenous oestradiol and progesterone can both cause increased uterine secretion and an increase in RNA polymerase activity in the nuclei of blastocyst cells within 48 h of injection (Moore, 1978; Shaw and Renfree, 1986). Similarly, exogenous steroids can reactivate tammar blastocysts and pregnancy proceeds to term (Berger and Sharman, 1969b; Renfree and Tyndale-Biscoe, 1973). Although both progesterone and oestradiol can initiate development, only progesterone will sustain it (Clark, 1968; Fletcher *et al.*, 1988).

An increase in uterine sensitivity to progesterone, resulting from either a change in progesterone receptor concentrations (see below) or from a change in a hormone which regulates uterine sensitivity to progesterone, could explain the apparent lack of a change in progesterone secretion rate (Shaw and Renfree, 1986). If prolactin regulates uterine sensitivity to progesterone as it does in the rabbit (Daniel, 1980; Daniel *et al.*, 1984), the removal of the inhibitory effect of sucking by day 3 RPY may allow a progestational response in the uterus before progesterone concentrations increase significantly (Shaw and Renfree, 1986).

The conclusion from the preceding section is that the first 6 days of blastocyst reacti-

vation is under maternal control (Figure 7.19).

The sucking stimulus must be withdrawn for 3 days (Gordon *et al.*, 1988), acting perhaps by failure of a single dawn pulse of prolactin on three successive occasions. The ovary (? corpus luteum only) must be present for more than 2 days but less than 4 (? 3) for reactivation to occur (Sharman and Berger, 1969; Berger, 1970). However, for maintenance of pregnancy ovarian input is required to day 6, to allow for the effects of the transient progesterone pulse on days 5–6 from a corpus luteum in which cell divisions are first seen at day 4 (Sharman and Berger, 1969). Changes in uterine secretion and uterine metabolism also occur by day 4 (Renfree, 1973b; Shaw and Renfree, 1986), and blastocyst metabolism has increased by day 5 (Moore, 1978; Thornber *et al.*, 1981; Shaw and

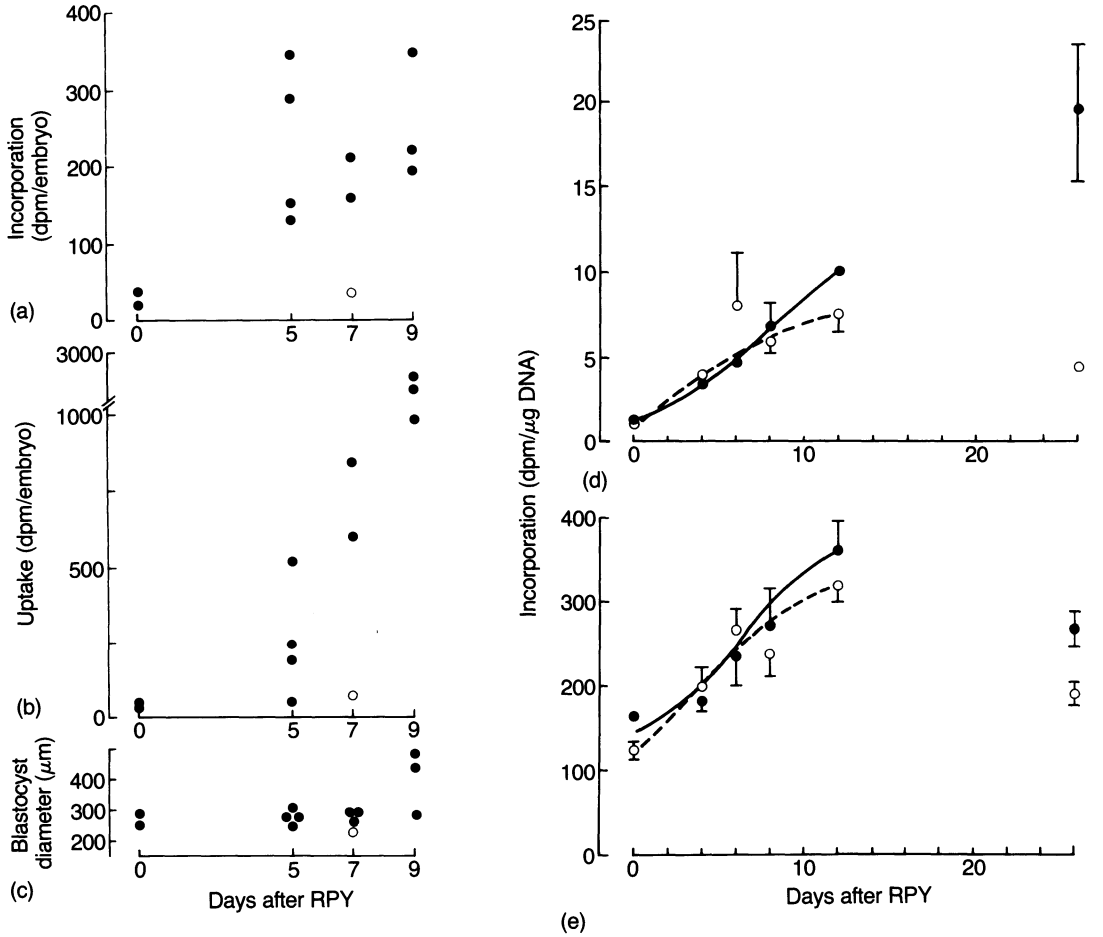


Figure 7.18 Uridine (a) incorporation and (b) uptake by individual blastocysts (d.p.m. per embryo) of the tammar during the early stage of reactivation. (○) Blastocyst with collapsed trophoblast. Blastocysts diameters are shown in (c). (d and e) Leucine incorporation by gravid (● — ●) and non-gravid (○ - - - ○) endometrium during pregnancy (d.p.m/μg of tissue DNA). Incorporation into (d) secreted protein and (e) tissue protein increased between day 0 and day 12. At day 26 gravid uteri were more secretory than non-gravid uteri. Tissue incorporation decreased between day 12 and day 26 in both uteri. Data derived from Shaw and Renfree (1986). (Redrawn from Shaw, 1983b.)

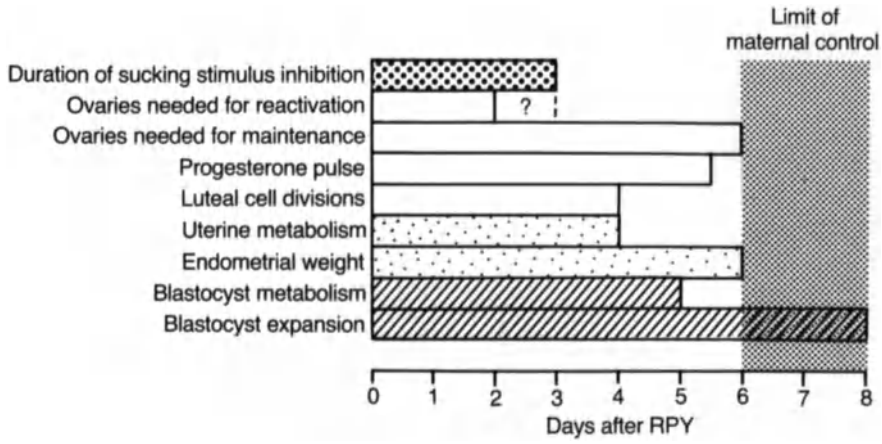


Figure 7.19 Diagrammatic representation of the timing of various maternal and embryo responses initiated by removal of the pouch young at day 0. The sucking stimulus must be withdrawn for 72 h to allow reactivation, and it appears that the ovaries must be present for all of this time. If they are removed at day 2, and probably day 3, reactivation is prevented, but at day 4 and day 6 reactivation occurs but pregnancy fails. The progesterone (and oestradiol) pulse occurs at days 5–6, after endometrial metabolism (day 4) and blastocyst metabolism (day 5) have already increased. The ovaries are necessary for a successful pregnancy only to day 8, as after removal between days 6 and 8 pregnancy can go to term in the absence of the ovary or the pituitary. Endometrial weight has significantly increased by day 6, and blastocyst diameter by day 8. Thus, the limit (earliest day 6; latest day 8) of the maternal influence on early pregnancy (stippling) is very brief, and the ovary and pituitary only become necessary again for the closing stages of pregnancy and parturition. Sucking pouch young effects ⊠; ovarian influence □; uterine responses ▩; embryo responses ▨. Based on data from various authors and see text for full references.

Renfree, 1986). The first gross morphological change, blastocyst expansion, is not seen until day 8 (Renfree and Tyndale-Biscoe, 1973) which is the limit of the maternal control as pregnancy can proceed to full term in the absence of ovaries, functional corpora lutea or pituitary after this time (Tyndale-Biscoe and Renfree, 1987; Tyndale-Biscoe and Hinds, 1989).

7.6.4 SEASONAL REACTIVATION

A great deal is now known of the endocrine control of seasonal reactivation in the tammar and in the Bennett's wallaby (Tyndale-Biscoe and Renfree, 1987). These two species are effectively pregnant for 364 of the 365 days of the year, the single non-pregnant day being the day it gives birth. In the normal course of events, tammar blastocysts reactivate shortly (about 20 days) after the summer solstice on December 22 (Figure 7.20), and it is now known that photoperiodic cues mediated via the pineal and its hormone melatonin are responsible for this (Renfree *et al.*, 1981; McConnell, 1984; McConnell *et al.*, 1986; Tyndale-Biscoe *et al.*, 1986), as it is in the Bennett's wallaby (Loudon *et al.*, 1985; Curlewis and Loudon, 1988, 1989; Loudon and Curlewis, 1989).

Since this has been reviewed extensively recently, and only indirectly relates to pregnancy (Tyndale-Biscoe *et al.*, 1986; Tyndale-Biscoe and Renfree, 1987), only a brief summary will be given here.

Manipulation of photoperiod has shown that seasonal quiescence is superimposed on the lactational inhibition of reproduction and

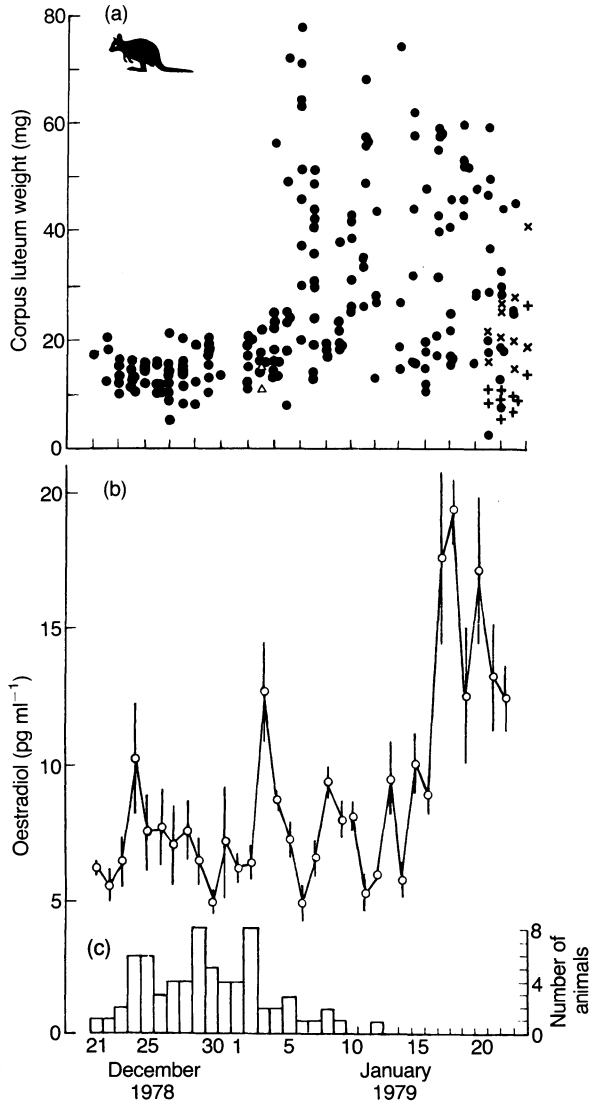


Figure 7.20 Changes at reactivation in 201 female *Macropus eugenii* shot between 20 December 1978 and 23 January 1979. (a) Weight of the corpus luteum of each animal, showing the onset of growth in early January, coincident with the expansion of the reactivated blastocyst. One animal killed on 2 January carried two blastocysts and two corpora lutea (Δ). In animals carrying newborn pouch young on or after 20 January, the corpora lutea of pregnancy are indicated by x and those resulting from the post-partum ovulation are shown by +. (b) Concentrations (mean ± SEM) of oestradiol-17–β in cardiac plasma from 143 pregnant and post-partum animals. Note the peak on 3 January and the longer period of elevated concentrations at the time of parturition and post-partum oestrus. Six of the animals sampled on 21 and 22 January carried newborn pouch young. (c) Date of reactivation calculated for 69 of these animals from the diameter of reactivated vesicles flushed from uteri between 1 and 20 January and crown–rump lengths of fetuses obtained between 14 and 22 January, using the growth curve (see Figure 7.13) derived from embryos reactivated after removing pouch young. Data from Flint and Renfree (1982). (From Tyndale-Biscoe and Renfree, 1987, with permission.)

that tammars experiencing increasing day length or long days have an inhibition of the corpus luteum (Sadleir and Tyndale-Biscoe, 1977; Hinds and den Ottolander, 1983; McConnell and Tyndale-Biscoe, 1985; McConnell *et al.*, 1986, Tyndale-Biscoe *et al.*, 1986). The evidence is that the pineal is transducing this photoperiodic information and the control of seasonal breeding through its secretion of melatonin, and the marked circadian fluctuations observed are abolished by denervation of the pineal, by pinealectomy, or by implants or injections of melatonin (Renfree *et al.*, 1981; McConnell, 1984; Renfree and Short, 1984; Loudon *et al.*, 1985; McConnell and Hinds, 1985; McConnell and Tyndale-Biscoe, 1985; Gemmell, 1987). As noted earlier, it appears that melatonin abolishes a marked pulse of prolactin of less than 2 h duration which coincides with the time of lights on (McConnell *et al.*, 1986), and that it does so within 3 days (Tyndale-Biscoe *et al.*, 1986). The abolition is permanent once the melatonin signal has been read, supporting the idea that melatonin does not affect the pituitary directly but operates through a neural mechanism in the hypothalamus (Renfree and Short, 1984; Tyndale-Biscoe *et al.*, 1986).

The time taken from a photoperiod change to birth or oestrus is longer than RPY to birth or oestrus, and yet with melatonin injections the prolactin pulse is abolished by the third day (Tyndale-Biscoe *et al.*, 1986), as in removal of the sucking inhibition (Gordon *et al.*, 1988), and if melatonin implants are given the shortest time to birth is identical to that of RPY to birth (Renfree and Short, 1984). Since the time from the progesterone pulse to birth is similar (melatonin treatment 21.6 days; photoperiod change 23.5 days; natural photoperiod change after summer solstice 22.0 days), the additional days before reaction must reflect the time taken for the message to be acted upon, and relate to how quickly and by how much the melatonin concentrations change (Tyndale-Biscoe *et al.*, 1986). Clearly these data provide new and exciting information on how seasonal breeding is controlled in mammals.

7.6.5 CONTROL OF DIAPAUSE IN MACROPODIDS

The control of diapause in macropodid marsupials is a complex interaction between the sucking stimulus, the hypothalamus, the pituitary, the corpus luteum and the uterus, resulting, in the case of the tammar, in an 11-month cessation of growth of the 100-cell blastocyst. Photoperiodic signals are superimposed on this system in the second half of the year when days are lengthening. Most of the steps on the hypothetical pathways have now been filled in (Figure 7.21), but the precise sequence of events between day 3 and day 6 after RPY needs further study.

It remains possible that the blastocyst can respond directly to pituitary or ovarian hormones, but Tyndale-Biscoe's (1970) elegant experiments transferring quiescent blastocysts to uteri of reactivated, ovariectomized recipients strongly suggest that the quiescent blastocyst does not require direct ovarian stimulation but simply awaits a changed uterine milieu. This is also further discussed below under 'The uterine endometrium' (section 7.7.3).

7.7 MAINTENANCE OF PREGNANCY

7.7.1 PITUITARY–OVARIAN INTERACTIONS

Pituitary – ovarian interactions are known for only one species, *M. eugenii* with additional unpublished results on the brush-tailed possum (see Hinds, 1983, 1988; Tyndale-Biscoe *et al.*, 1986; Tyndale-Biscoe and Renfree, 1987; Tyndale-Biscoe and Hinds, 1989). The marsupial corpus luteum does not appear to depend on a luteotrophic stimulus for progesterone secretion, nor is its activity determined by luteolysins. In *M. eugenii* the previously formed corpus luteum undergoes

its normal growth and secretory functions after total hypophysectomy (Hearn, 1974; Tyndale-Biscoe and Hawkins, 1977; Hinds, 1983).

Pregnancies also go to term in hypophysectomized tammars but the fetuses die in the uterus (Hearn, 1973, 1974). A similar independence of the corpus luteum from the pituitary is observed in brush-tailed possums after hypophysectomy, so it seems that the

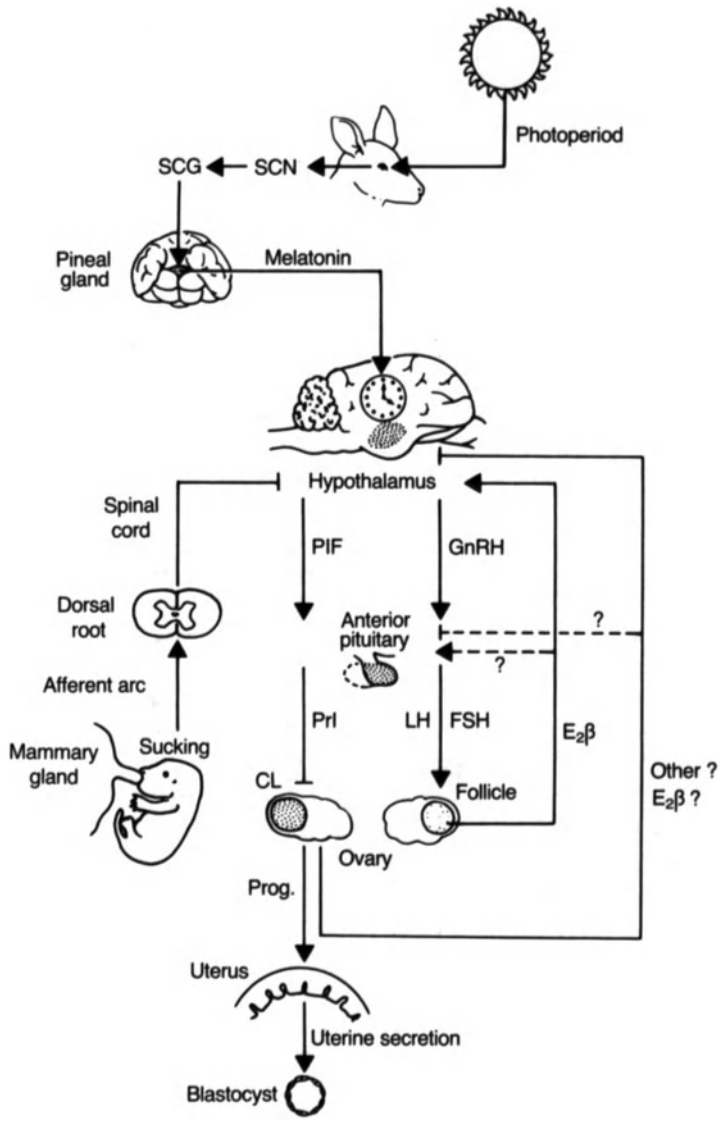


Figure 7.21 Hypothetical scheme showing the known and suggested pathways for the control of quiescence of *Macropus eugenii*. CL, corpus luteum; GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; $E_2\beta$, oestradiol-17β; FSH, follicle-stimulating hormone; Prog, progesterone; PIF, prolactin inhibiting factor; Prl, prolactin; SCG, superior cervical ganglion; SCN, suprachiasmatic nucleus. ↓ Stimulation; ⊥ inhibition. (Redrawn from Renfree, 1981b.)

autonomy of the corpus luteum may be a common feature of marsupials (Tyndale-Biscoe *et al.*, 1986). However, functional corpora lutea and successful births do occur in tammars passively immunized to GnRH (Short *et al.*, 1985), confirming that FSH and LH are not required for luteal reactivation, pregnancy, parturition or lactogenesis in the tammar, but that follicular growth and postpartum oestrous behaviour are inhibited in the absence of gonadotrophins. Hypophysectomy performed at any stage of the oestrous cycle or pregnancy prevents growth of follicles (Hearn, 1974; Panyaniti *et al.*, 1985). Using heterologous radioimmunoassays, two gonadotrophic fractions which resemble FSH and LH of eutherian mammals have been described (Gallo *et al.*, 1978), and LH remains low (0.2–1.9 ng/ml) at all times during the oestrous cycle or pregnancy except for the pre-ovulatory surge of 10–50 ng/ml, which lasts for about 12 h (Sutherland *et al.*, 1980; Tyndale-Biscoe *et al.*, 1983). In intact females FSH is not detectable (Evans *et al.*, 1980), although it is detectable in intact males (Catling and Sutherland, 1980), but it reaches very high concentrations in castrated males (Catling and Sutherland, 1980) and ovariectomized females 3–8 days after ovariectomy (100–600 ng/ml) (Evans *et al.*, 1980; Horn *et al.*, 1985). As in eutherian mammals, gonadotrophins are controlled by oestradiol concentrations, with low doses exerting a negative feedback, but high doses, similar to the levels which occur at oestrus, exerting a positive feedback on the hypothalamus to induce a transient pulse of LH (Horn *et al.*, 1985). The corpus luteum also provides a negative feedback on the pituitary (Tyndale-Biscoe and Hawkins, 1977; Evans *et al.*, 1980; Tyndale-Biscoe and Hearn, 1981; Renfree *et al.*, 1982) to suppress ovulation, apparently by the secretion or release of oestradiol (see Figure 7.21) and, although as yet no evidence can be found for oestradiol-17β secretion by the tammar corpus luteum *in vitro* (Renfree *et al.*, 1984b), there is now evidence of oestradol secretion by the corpus luteum of the quokka *Setonix brachyurus* (Bradshaw and Bradshaw, 1992).

By contrast, ovarian cortical tissue does secrete oestradiol (Renfree *et al.*, 1984b) and has a negative-feedback effect on the pituitary (Tyndale-Biscoe and Hearn, 1981).

7.7.2 THE CORPUS LUTEUM

Development of the embryo past the unilaminar blastocyst stage begins with expansion of the yolk sac to form first a bilaminar and then a trilaminar vesicle. In all the species of which we have some knowledge of the hormonal profile, progesterone increases in concentration and the endometrium proliferates. Although the importance of luteal secretion is unequivocal, the need for it is relatively brief, and it is during the first week of pregnancy that it is most important. Ovariectomy or lutectomy of the tammar, brush-tailed possum, potoroo, quokka and opossum demonstrates that fetal development can proceed to full term in the absence of the corpus luteum after 8–10 days (Hartman, 1925; Tyndale-Biscoe, 1963a, 1970; Sharman, 1965b; Sharman and Berger, 1969; Shorey and Hughes, 1973a,b, 1975; Renfree, 1975; I.R. Young and Renfree, 1979; Bryant and Rose, 1986).

The endocrine role of the corpus luteum during pregnancy and the cycle has been best defined in macropodids, and in the tammar wallaby in particular (Tyndale-Biscoe, 1984; Tyndale-Biscoe and Renfree, 1987). Progesterone has been identified as the main steroid in corpora lutea of the tammar (Renfree *et al.*, 1979; Renfree *et al.*, 1984b), reaching maximum concentrations of 32 ng/mg at day 22 of the 27-day gestation when the corpus luteum weighs around 60 mg (Figure 7.22).

The pattern of circulating plasma progesterone is similar in the macropodid species so far studied. During lactational or seasonal quiescence, peripheral plasma concentrations are low, around 100–200 pg/ml,

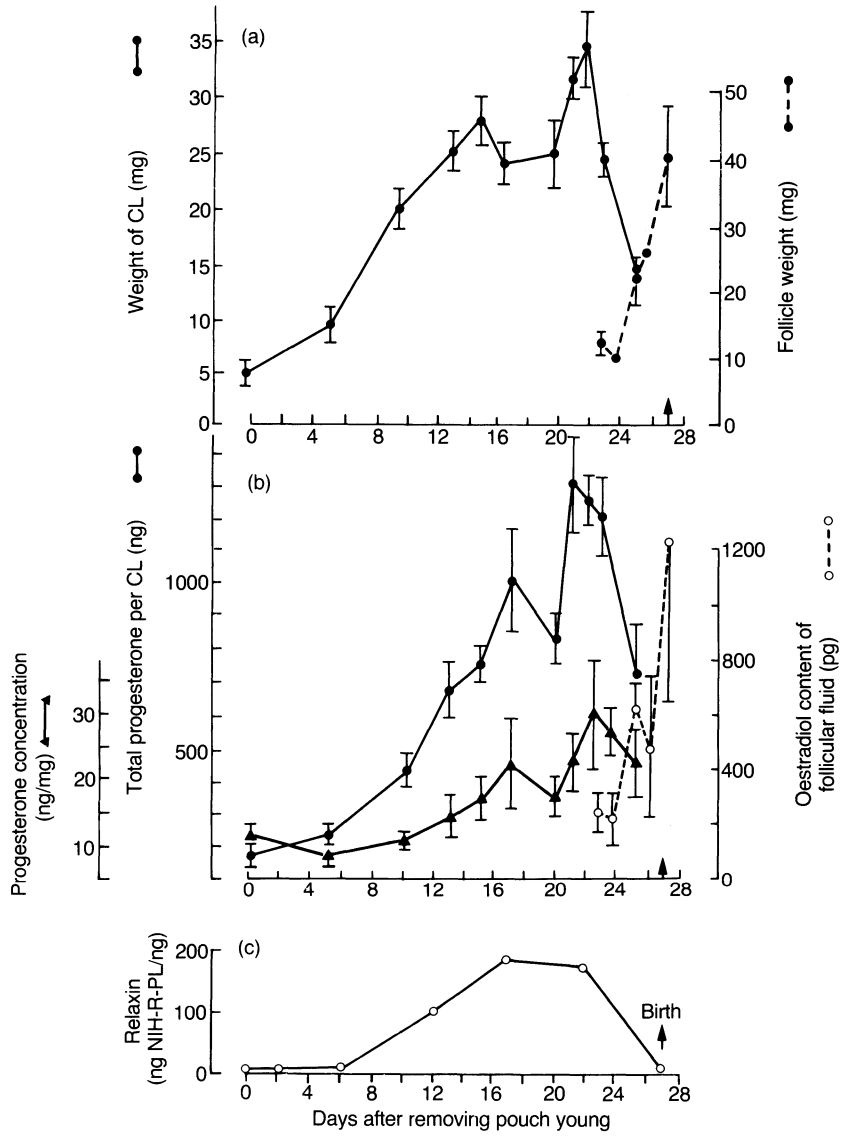


Figure 7.22 Hormonal content of the corpus luteum and follicle in the tammar during pregnancy and immediately post partum. (a) Wet weight (mg) ± SEM of the corpus luteum and follicle. (b) Progesterone concentration (△) and content (•) of the corpus luteum and oestradiol content (○) of the follicle. (c) Relaxin concentration of the corpus luteum. Progesterone and corpus luteum weight data from Renfree *et al.* (1979); oestradiol and follicle weight data from Harder *et al.* (1985); relaxin data from Tyndale-Biscoe (1981). Steroids were measured by radioimmunoassay; relaxin by the mouse pubic symphysis bioassay agonist ng NIH-R-PL/mg relaxin standard.

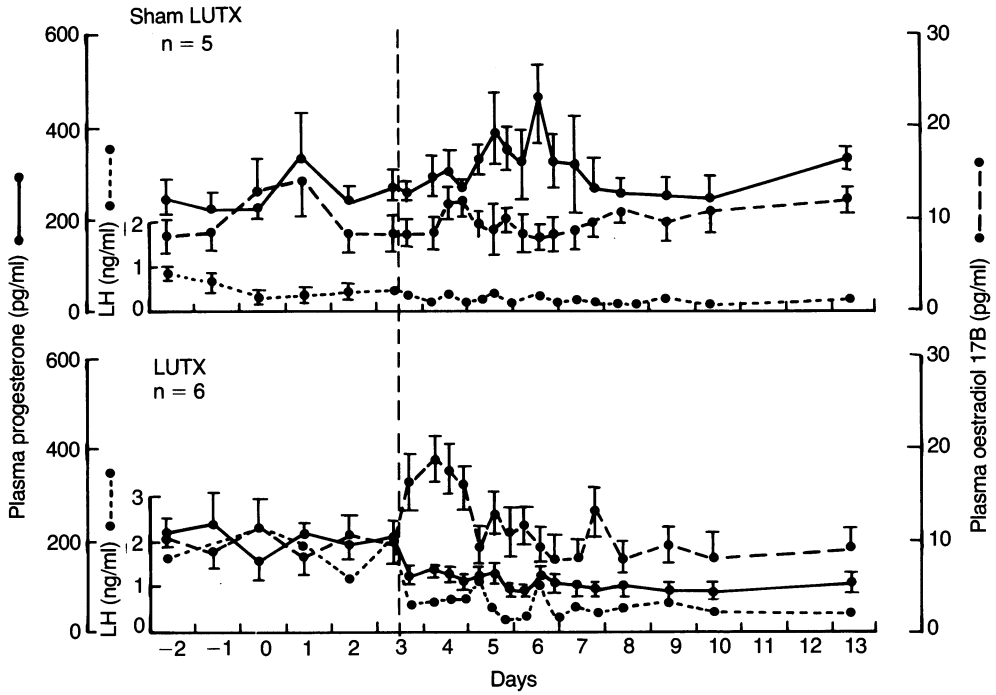


Figure 7.23 Plasma concentrations of progesterone (● — ●), oestradiol-17β (● - - - ●) and LH (NIH-oLH-23) (● . . . ●) in tammars before and after excision of the corpus luteum (n=6) and sham excision (n=5) of the corpus luteum reactivated by removal of the pouch young (RPY) on day 0. The time of operation is indicated by the vertical line. Values are mean ± SEM; error bars are not shown when less than the size of the symbol. Data from Fletcher and Renfree (1988).

rising to a transient early pulse which occurs at day 3–4 in the quokka and day 4, 5 or 6 in the tammar and Bennett's wallaby (Lemon, 1972; Renfree and Heap, 1977; Cake *et al.*, 1980; Hinds and Tyndale-Biscoe, 1982a; Walker and Gemmell, 1983a) after reactivation of the diapausing blastocyst. Peak concentrations are reached in late pregnancy followed by a precipitous fall at the time of parturition (Figures 7.22 and 7.27).

Follicular size begins to increase by about day 23 RPY, reaching the maximum at around the time of birth (Figure 7.22). Oestradiol content of the follicular fluid also increases to reach a peak post-partum (Harder *et al.*, 1985) and peripheral plasma levels remain relatively constant throughout most of pregnancy (Harder *et al.*, 1984; Shaw and Renfree, 1984). However, there is also a brief pulse of oestradiol around day 5, coincident with the progesterone pulse (Shaw and Renfree, 1984; Figure 7.5). Like the progesterone pulse, it comes too late to be involved in the resumption of development after diapause, but may be important for providing some stimulus to the endometrium.

The synthesis of progesterone by the corpus luteum increases at day 5 *in vitro* (Hinds *et al.*, 1983), and *in vivo* lutectomy experiments have shown that the pulse is of luteal origin (Fletcher and Renfree, 1988). Although the corpus luteum does contain oestrogens (Renfree *et al.*, 1984b), the oestradiol pulse occurs even after lutectomy (Figure 7.23).

The origin and function of this transient rise in oestrogen remain to be determined. It is interesting to note, however, that a transient early rise in oestradiol is also seen in wild tammars at the time of natural reactivation after the summer solstice (Flint and Renfree, 1982; Figure 7.20).

The corpus luteum is also a source of relaxin. Tyndale-Biscoe (1966, 1969, 1981) has measured relaxin using the mouse pubic symphysis bioassay in the brush possum and in the tammar. In the tammar the maximal response in corpora lutea occurs between days 16 and 23 RPY, with a marked fall postpartum (Figure 7.22). The possible significance of this will be discussed further in the section on parturition (section 7.9).

Following the early transient pulse in progesterone in macropodids there is a steady rise in progesterone, reaching a peak by mid-pregnancy at around the time the shell membrane attenuates and dissolves (Renfree, 1977; Denker and Tyndale-Biscoe, 1986) which allows the expanded yolk sac trophoblast to make its first direct contact with the uterine endometrium. The corpus luteum is the source of this mid-pregnancy progesterone, for lutectomy at day 18 RPY (Findlay *et al.*, 1983) or day 23 RPY (Harder *et al.*, 1984) causes an immediate drop in plasma levels to those seen during quiescence (Figure 7.24).

Concentrations of progesterone remain high until they fall dramatically at parturition (Figure 7.22). In most non-macropodid species, a similar pattern of luteal growth and progesterone secretion is seen, but without the early pulse (Figures 7.25 and 7.28) but in the bandicoots parturition occurs during the peak of the luteal phase and corpora lutea remain large and secrete progesterone for most of lactation (Figure 7.26).

Thus from structural and endocrine studies three broad patterns of corpus luteum growth and decline have been recognized (Table 7.3) by Tyndale-Biscoe and Renfree (1987); and the following account is derived from their classification.

These types correlate with the reproductive strategies (groups 1–4) outlined earlier (see section 7.2).

Type 1: short gestation, short luteal phase;
Type 2: short gestation, prolonged luteal phase; and
Type 3: long gestation, long luteal phase.

No data are available for group 4 animals, which include the small possum families. In the type 1 pattern, which probably holds for the majority of marsupials, the luteal phase occupies less than 60% of the oestrous cycle and is followed by a follicular phase leading

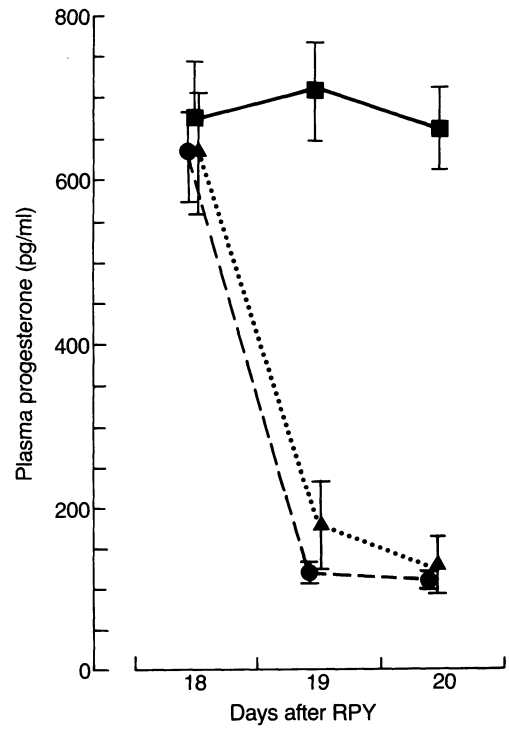


Figure 7.24 Plasma progesterone before and 24 and 48 h after surgery to excise the corpus luteum on day 18 after removal of pouch young (RPY). Values are means ± SEM of pregnant lutectomized tammars (n=6) (●), pregnant sham-operated animals (n=5) (■), and non-pregnant lutectomized animals (n=4 at day 18 and 19, n=3 at day 20 after RPY (▲). (Redrawn from Findlay *et al.*, 1983.)

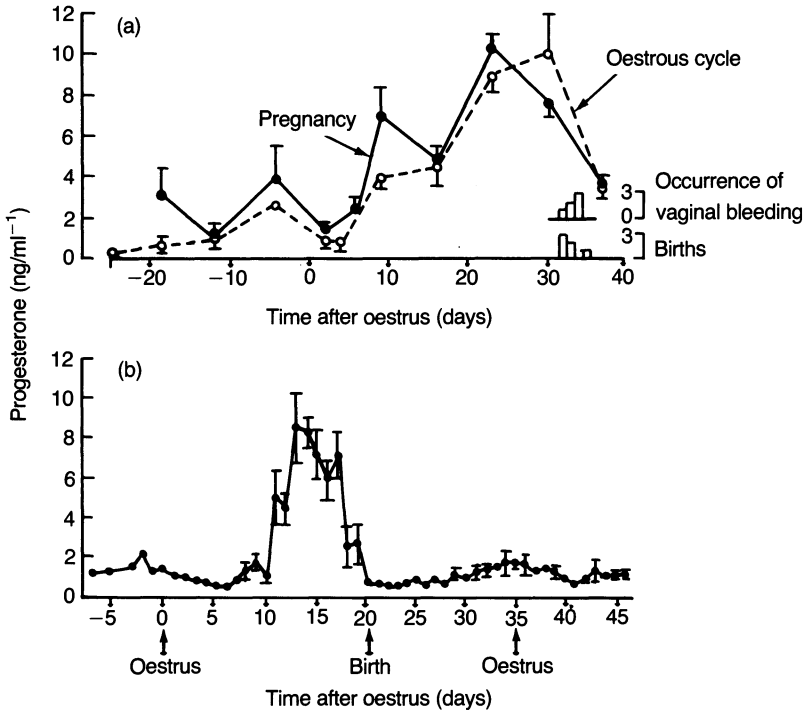


Figure 7.25 Plasma progesterone concentrations in two dasyurids during an oestrous cycle. (a) Summary of changes in a group of six *Dasyuroides byrnei* which underwent an oestrous cycle (broken line) followed by a pregnancy (solid line). Concentration (mean ± SEM) of plasma progesterone to show sharp drop before birth, or vaginal bleeding in non-pregnant cycle. All values were synchronized to day 2 and correlated with the uterine cell cycle, mating and birth. Data from Fletcher (1983, 1989b). (b) Concentration (mean ± SEM) of plasma progesterone in *Dasyurus viverrinus* through the oestrous cycle. The time that birth would occur in a pregnant animal is shown. Data from Hinds (1983, 1989a). (a and b from Tyndale-Biscoe and Renfree, 1987, with permission).

to the next oestrus and ovulation. The duration of pregnancy is shorter than the luteal phase (see Figure 7.2), and parturition coincides with corpus luteum regression. If lactation follows, the subsequent follicular phase is suppressed and the corpus luteum of pregnancy slowly disappears or remains as a corpus albicans. In the type 2 pattern, as exemplified by the bandicoots, gestation is equally short but parturition occurs during the peak of the luteal phase. If lactation follows, the corpora lutea remain large and the follicular phase is suppressed for most of lactation. In the type 3 pattern, which occurs in most of the Macropodidae, the luteal phase lasts for more than 90% of the cycle and the follicular phase is not inhibited (see Figure 7.2). Gestation occupies all of the extended luteal phase and is followed by post-partum oestrus and ovulation. If lactation follows, the corpus albicans associated with pregnancy declines further, as in the non-pregnant female, but the new corpus luteum formed at post-partum ovulation is held in a state of quiescence whilst the young is sucking, and in the tammar and Bennett wallabies for some months afterward. In all types, the rise in peripheral progesterone appears to be important for the full development of the secretory endometrium, which in

turn supports the rapid expansion of the embryo vesicle.

7.7.3 THE UTERINE ENDOMETRIUM

During pregnancy the endometrium proliferates and becomes secretory. In the monovular grey kangaroo, *M. giganteus*, R. Owen (1834) was the first to note that there were differences between the endometrium of the gravid and that of the contralateral non-gravid, uterus, a finding with which Flynn (1930) concurred in his study of the Tasmanian bettong, *Bettongia gaimardi*. Because of the similarity of the changes in the uterus during pregnancy and the oestrous cycle, these observations were largely overlooked until it was found that the wet weight of the gravid endometrium of the tammar was consistently and significantly greater than that of the adjacent, non-gravid, one (Renfree, 1972a; Renfree and Tyndale-Biscoe, 1973). The composition of the uterine secretions also differed, not only throughout pregnancy but between the two sides (Renfree, 1973b). The rate of protein synthesis is greater in the gravid uterus (Shaw and Renfree, 1986).

These observations of a unilateral effect suggested that the presence of the fetus or placenta may be responsible, and this has been confirmed in three ways. First, the

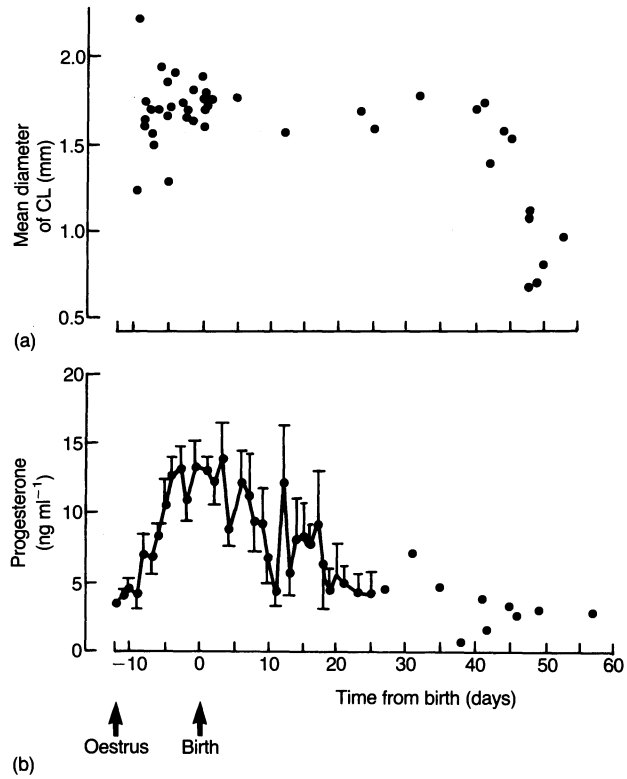


Figure 7.26 (a) Diameter of the corpus luteum (CL) in the bandicoot *Isoodon macrourus* during pregnancy (between arrows) and the subsequent lactation. During the 45–53 days of lactation, ovulation may recur. Data from Lyne and Hollis (1979). (b) Concentrations (mean ± SEM) of plasma progesterone in *Isoodon macrourus* during pregnancy and lactation. Individual samples after day 25 are shown. Data from Gemmell (1981). (From Tyndale-Biscoe and Renfree, 1987, with permission).

Table 7.3 Marsupial species for which plasma or luteal hormonal measurements are available during **pregnancy**, arranged using the classification of reproductive patterns of Tyndale-Biscoe and Renfree (1987). Nothing is known of the hormones in species with group 4 pattern (Families Tarsipedidae, Burramyidae, Acrobatidae)

Type and species	*Hormones measured*	*References*
(a) Short gestation, short luteal phase		
Didelphis virginiana	P, E	Cook and Nalbandov (1968); Cook *et al.* (1977); Harder and Fleming (1981)
Dasyurus viverrinus	P	Hinds (1983, 1989c)
Dasyuroides byrneii	P, LH	Fletcher (1983, 1989a,b)
Antechinus stuartii	P	Hinds and Selwood (1990)
Trichosurus vulpecula	P, E, PGFM, LH, relaxin	Tyndale-Biscoe (1966, 1969); Thorburn *et al.* (1971); Shorey and Hughes (1973a,b, 1975); Hinds (1983); Curlewis *et al.* (1985); Curlewis and Stone (1986); Gemmell *et al.* (1987); C.A. Horn, unpublished, in Tyndale-Biscoe and Renfree (1987)
(b) Short gestation, prolonged luteal phase		
Isoodon macrourus	P, PGFM	Gemmell (1979, 1981, 1984); Gemmell *et al.* (1980)
(c) Long gestation, delayed luteal phase		
Macropus eugenii	P, E, PGFM, LH, FSH, Prl, relaxin, oxytocin	See footnote a for these references
Macropus rufogriseus	P, PGFM, Prl	Walker *et al.* (1983); Walker and Gemmell (1983a); Curlewis *et al.* (1986, 1988); Loudon *et al.* (1990)
Macropus giganteus	P, E, LH, Prl	C.H. Tyndale-Biscoe, L.A. Hinds and T.P. Fletcher, unpublished, in Tyndale-Biscoe and Renfree (1987)
Macropus rufus	P	Lindner and Sharman (1966)
Setonix brachyurus	P	Cake *et al.* (1980)

[a] Lemon (1972); Hearn (1974); Renfree and Heap (1977); Renfree *et al.* (1979); Evans *et al.* (1980); Heap *et al.* (1980); Sutherland *et al.* (1980); Tyndale-Biscoe (1981); Tyndale-Biscoe and Hearn (1981); Callard *et al.* (1982); Flint and Renfree (1982); Hinds and Tyndale-Biscoe (1982a,b); Renfree *et al.* (1982); F. Stewart & Tyndale-Biscoe (1982); Findlay *et al.* (1983); Hinds (1983, 1988); Hinds *et al.* (1983); Shaw (1983a,b); Tyndale-Biscoe *et al.* (1983); Harder *et al.* (1984, 1985); Renfree *et al.* (1984b); Shaw and Renfree (1984); K.L. Ward and Renfree (1984); Short *et al.* (1985); Lewis *et al.* (1986); Tyndale-Biscoe *et al.* (1988); Curlewis *et al.* (1988); Fletcher and Renfree (1988).

P, progesterone; E, oestradiol-17β; PGFM, prostaglandin $F_{2\alpha}$ metabolite; LH, luteinizing hormone; FSH, follicle-stimulating hormone; Prl, prolactin.

transfer of a blastocyst to a cyclic, non-pregnant animal, or to the side contralateral to the corpus luteum in a pregnant one, initiated similar endometrial proliferation in each uterus in which a fetus developed and did not relate to the position of the corpus luteum, so the greater weight was not due to the proximity of the corpus luteum of pregnancy (Renfree, 1972a; Renfree and Tyndale-Biscoe, 1973). In animals in which blastocyst reactivation was initiated during seasonal quiescence with exogenous progesterone, endometrial growth was induced in both uteri for the duration of treatment. However, after the injections ceased, only the uterus containing the developing embryo continued

to enlarge while the non-gravid uterus declined to a size usually observed in the non-pregnant cycle (Renfree and Tyndale-Biscoe, 1973). Since, in this experiment, the corpus luteum was by-passed and remained inactive, the continuation of endometrial stimulation in the gravid uterus after progesterone withdrawal again must have been due to the presence of the fetus or its placenta. The third observation suggests that the placenta is the main site of the influence. In some females which were treated with progesterone during seasonal quiescence to induce blastocyst reactivation, a vesicle grew but no embryo or vascular mesoderm developed (Renfree and Tyndale-Biscoe, 1973; Fletcher *et al.*, 1988), but these vesicles, consisting only of the non-vascular yolk sac membrane, stimulated the endometrium and the uterine secretions as in a normal pregnancy.

The fact that the endometrium of the non-gravid uterus declines in weight and secretory activity during the second half of pregnancy or the cycle, even though peripheral progesterone concentrations are at their highest (Figure 7.27) suggests that there is a declining sensitivity to progesterone.

It is possible that the local stimulation of the gravid uterus may reverse this by stimulating increased progesterone receptor synthesis but there is no evidence for this (D. Blanden and M.B. Renfree, unpublished) (section 7.7.4). F.J. Owen *et al.* (1982) have observed a difference in progesterone receptor concentration between the two uteri of the quokka *S. brachyurus*, an observation also made in the tammar (D. Blanden and M.B. Renfree, unpublished). A similar unilateral response of the endometrium to pregnancy has been observed in the quokka (Wallace, 1981) and also in the potoroo *Potorous tridactylus* by Shaw and Rose (1979). So far these effects are only known in macropodids; in non-macropodids, such as the monovular brush-tailed possum *Trichosurus vulpecula* and the polyovular opossum *Didelphis virginiana*, this fetal stimulation of the endometrium is not seen (Figure 7.28).

In opossums, uteri endometrium from equivalent days of the oestrous cycle and gestation are virtually identical in size, appearance and biochemical composition (Hartman, 1923; Renfree, 1975; Fleming and Harder, 1981a,b). Likewise, in the monovular brush-tailed possums, adjacent gravid and non-gravid uteri are identical except for the better development of the subepithelial capillary bed in the gravid uterus (Pilton and Sharman, 1962) and have similar endometrial weights (N.J. Cantrill and C.H. Tyndale-Biscoe, personal communication).

In both these species, maintenance of gestation depends on a continued uterine secretory phase. Ovariectomy of opossums between days 6 and 8 of the 12½-day gestation allows development of normal-term fetuses, but parturition is inhibited (Hartman, 1925; Renfree, 1974). In possums the uterine luteal phase develops by day 7 of the 17-day gestation (Pilton and Sharman, 1962; Shorey and Hughes, 1973a,b, 1975). After ovariectomy on day 7 the uterine secretory phase continues for the normal duration, but the volume of secretion is reduced (Shorey and Hughes, 1975). Ovariectomy on day 7 or 10 does not interfere with development to term of intrauterine embryos, but the animals do not give birth, while lutectomy on day 11 or injections of progesterone for 2 or 3 days after ovariectomy on day 7 results in some live births (Smith and Sharman, unpublished, in Sharman 1965b; and in Tyndale-Biscoe, 1966). Possums and opossums differ from eutherian mammals in which embryonic development can continue for several days after ovariectomy in that, because of their short gestation, even a brief survival after the operation allows them to develop to full term. Tammars and quokkas, despite their longer gestation, are able to extend the uterine secretory phase, so that they can develop to term after ovariectomy as early as day 8 or 7 respectively. Although the fetus or

its membranes appear to be involved, how they achieve this is still unclear, but both uterine steroid receptors and endocrine activity by the placenta are likely candidates.

7.7.4 PROGESTERONE AND OESTROGEN RECEPTORS IN THE FEMALE GENITAL TRACT

Very few attempts have been made, as yet, to measure ovarian steroid hormone receptors in target cells of marsupial genital tract tissue. So far, steroid receptors have been measured in only three species: the quokka (F.J. Owen *et al.*, 1982), the brush-tailed possum (C.E. Young and McDonald, 1982; Curlewis and Stone, 1986) and the tammar (D. Blanden and M.B. Renfree, unpublished). Evidence for the binding of progesterone is also found in the quoll, *Dasyurus viverrinus*, in addition to these three marsupials (T.P. Fletcher and

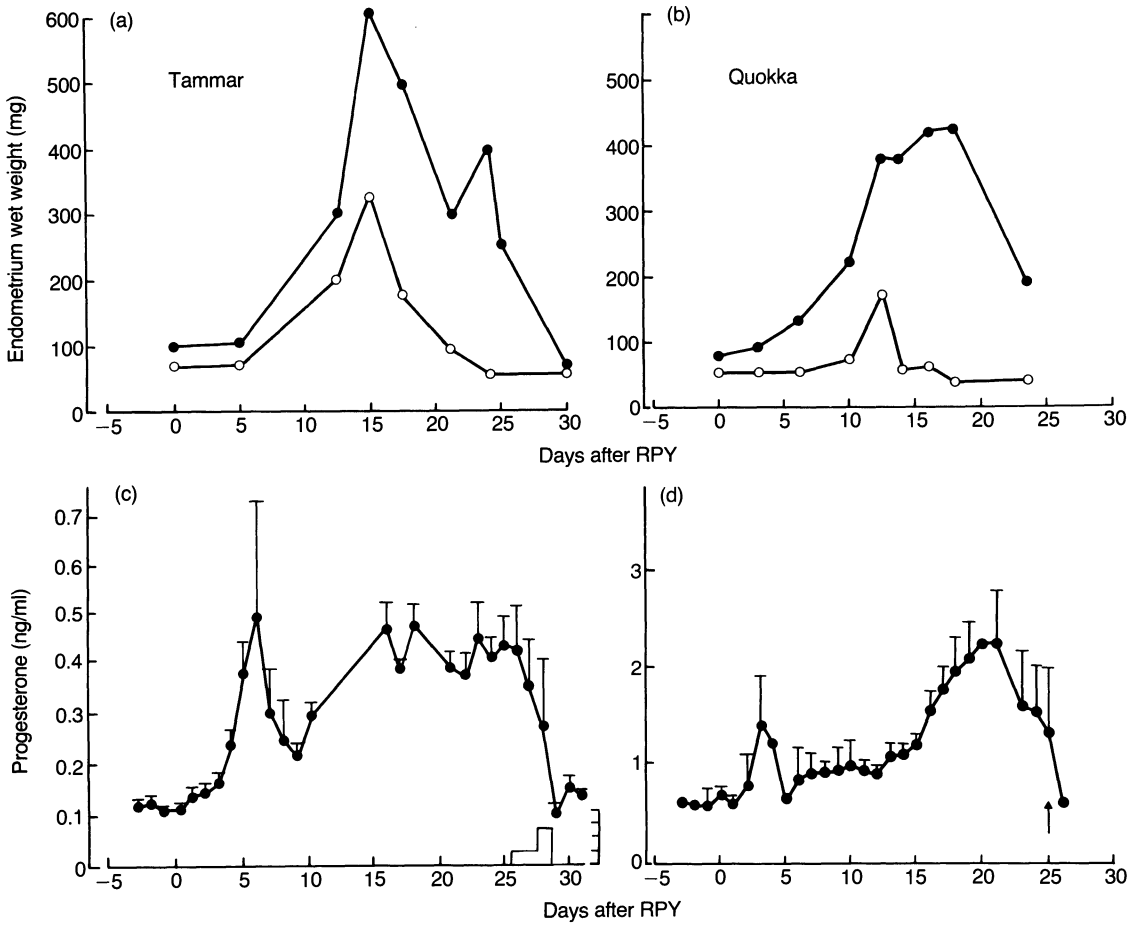


Figure 7.27 Endometrial wet weights (a and b) and plasma progesterone (c and d) in two macropodids, the tammar, *Macropus eugenii* (a and c), and quokka, *Setonix brachyurus* (b and d), during pregnancy. Gravid uteri (•) show a greater and more sustained response than contralateral, non-gravid uteri (○). Plasma progesterone measured by radioimmunoassay after removal of pouch young. Note both species show a marked pulse of progesterone 3–6 days after removal of their pouch young, and a sharp fall in progesterone at the time of parturition. [Redrawn from (a) Renfree and Tyndale-Biscoe (1973); (b) G.I. Wallace, unpublished observations, and Renfree (1980c); (c) Cake *et al.* (1980) and (d) Hinds and Tyndale-Biscoe (1982a).]

D.R. Blanden, personal communication). The properties of the possum cytosol oestrogen receptors in the endometrium and vaginal endothelium are similar to those described for eutherians, but differ in their relative binding affinities (C.E. Young and McDonald, 1982). Two distinct classes of high binding affinity for oestrogens were detected, with high (8S) and low (4S) molecular weight, the proportions of which varied with reproductive state or hormone treatment. Animals treated with oestrogen, or those in the follicular phase of the cycle had a significant increase in the concentration of macromolecular high-affinity oestrogen binding sites in the endometrium (predominantly 8S) and even more so in the vaginal endothelium (mainly 4S during pregnancy and the luteal phase) (C.E. Young and McDonald, 1982). During the non-pregnant cycle, cytosol oestradiol receptor concentrations rose between days 0 and 5, after which they declined

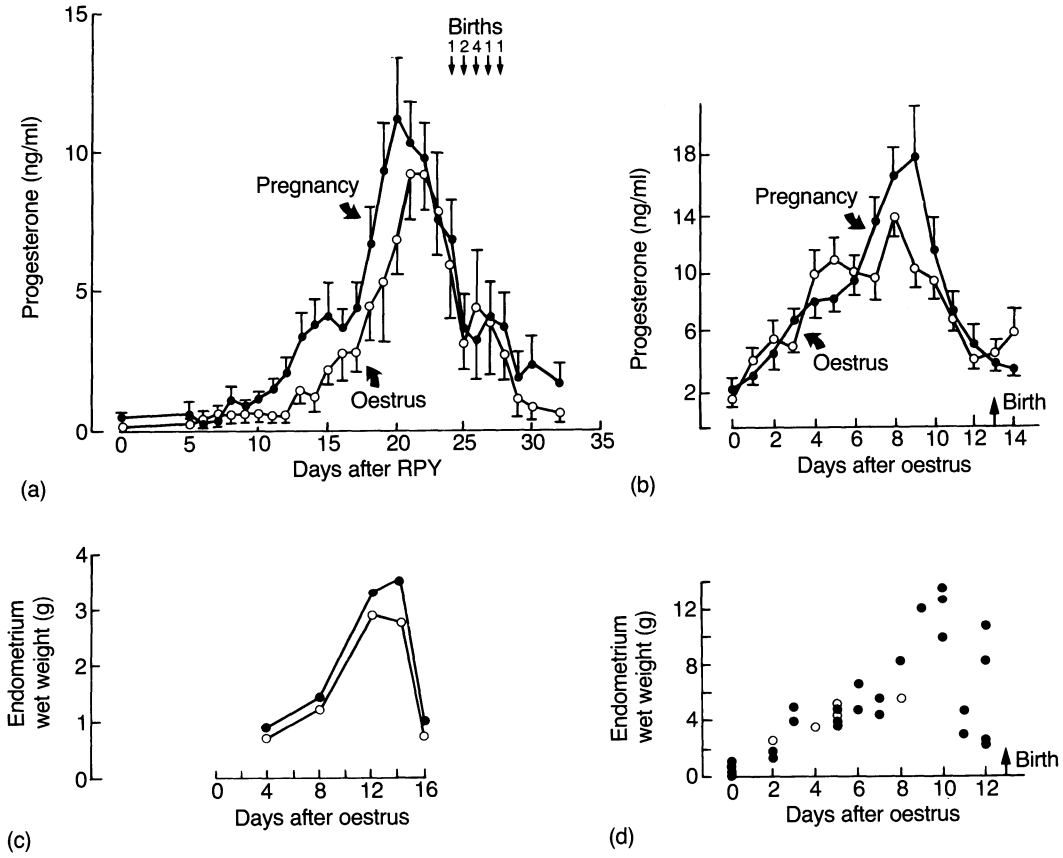


Figure 7.28 Plasma progesterone (a and b) concentrations during pregnancy (•) and the oestrous cycle (○) in brush-tailed possum, *Trichosurus vulpecula* (a), and the opossum, *Didelphis virginiana* (b). Endometrial wet weights in gravid (•) or non-gravid (○) uteri of both possums (c) and opossums (d) reflect the increasing progesterone concentrations. In the monovular brushtail possum there are no unilateral effects of pregnancy. In the polyovular opossum both uteri are normally gravid; non-gravid uteri (○) are from cycling animals at differing days after oestrus. [Redrawn from (a) Gemmell *et al.* (1987); (b) Harder and Fleming (1981); (c) N.J. Cantrill and C.H. Tyndale-Biscoe, unpublished results and (d) Renfree (1975).]

(Curlewis and Stone, 1986). In ovariectomized animals oestradiol increased the uterine weight, RNA–DNA and protein–DNA ratios and the concentration of cytosol receptors for oestradiol and progesterone (Curlewis and Stone, 1986). Thus, both studies show that oestradiol cytosol receptor levels in the uterus of the brush-tailed possum are high at oestrus and low during the luteal phase.

Progesterone receptors have been detected in uterine tissue of the quokka, and exogenous oestradiol leads to an augmentation of the uterine receptor concentration, with a six-fold increase after 5 days of treatment (F.J. Owen *et al.*, 1982). It appears that the level of oestrogen in the circulation influences the responsiveness of the uterus to progesterone, and within an hour of oestrogen priming there is a depletion of cytosolic progesterone binding and increased nuclear localization (F.J. Owen *et al.*, 1982). In the brush-tailed possum, progesterone receptor levels are highest at day 0 of the cycle, after which they decline to day 13 (Curlewis and Stone, 1986) (Figure 7.29).

Progesterone receptors in this species are low after ovariectomy, but, as in the quokka, increase in concentration after oestradiol treatment (Curlewis and Stone, 1986). The decline in progesterone cytosol receptor levels after oestrus is similar to that seen in many eutherian species during the phase of progesterone dominance (Curlewis and Stone, 1986).

A much more extensive series of measurements has been made on the steroid receptors throughout pregnancy in the tammar, and an interesting pattern is emerging (D. Blanden and M.B. Renfree, unpublished). As we found for endometrial wet weight, there is a unilateral effect throughout pregnancy for the concentration of both progesterone and oestradiol receptors, with greater concentrations in the gravid uterus, and there is also a peak concentration of both receptor concentrations at day 5 of pregnancy after RPY, coinciding with the peripheral plasma

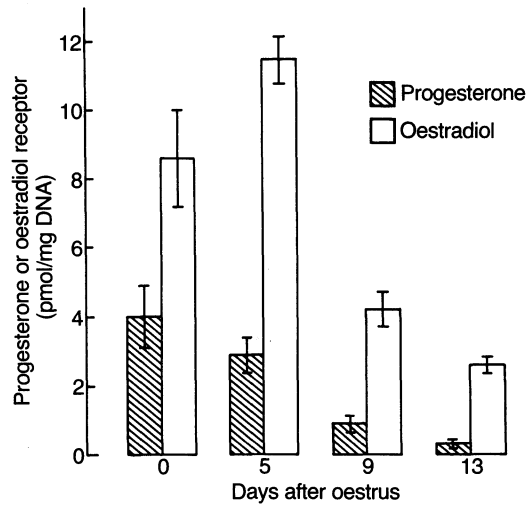


Figure 7.29 Concentrations of progesterone and oestradiol receptor during the oestrous cycle in the brush-tailed possum. It is interesting that there is a marked peak in oestradiol receptor concentrations 5 days post oestrus, while that of progesterone declines steadily throughout the cycle. Data from Curlewis *et al.* (1985).

peaks of oestrogen (Shaw and Renfree, 1984) and progesterone (Hinds and Tyndale-Biscoe, 1982a). Myometrial receptor concentrations mirror those of the endometrium although they are generally lower. Concentrations of progesterone receptor in the lateral vaginae are almost unmeasurable, while the oestrogen cytosol receptor concentrations are lower than those of the myometrium.

7.7.5 ENDOCRINE FUNCTIONS OF THE PLACENTA

The morphogenetic changes just described appear to be associated with the presence of the placenta, and it could provide a local endocrine stimulation to the adjacent uterus without any hormone being detected in the peripheral circulation. This could explain why pregnancy continues to full term after ablation of the corpus luteum or ovariectomy

in several marsupials and there is now evidence that the placenta is far from inactive. The yolk sac placenta is only three cell layers thick in the vascular region, and is bilaminar for over half of its surface (Figure 7.30).

Incubations of the meagre amount of placental tissue available (< 100 mg) from the quokka *S. brachyurus* and the tammar *M. eugenii* have shown that they have incipient endocrine activity by converting pregnenolone to progesterone (Bradshaw *et al.*, 1975; Renfree and Heap, 1977; Renfree, 1977). A more detailed study of the yolk sac membrane of *M. eugenii* showed that it can convert a range of steroid precursors into a variety of products (Figure 7.30), although levels of conversion were generally low (Heap *et al.*, 1980). Small amounts of prenenolone were converted to progesterone and pregnanediol; androstenedione was metabolized to 5α-androstane-3,17-dione and androsterone, but was not incorporated into oestrogens, providing no evidence for the enzymes arylsulphatase or sulphotransferase in the pla-

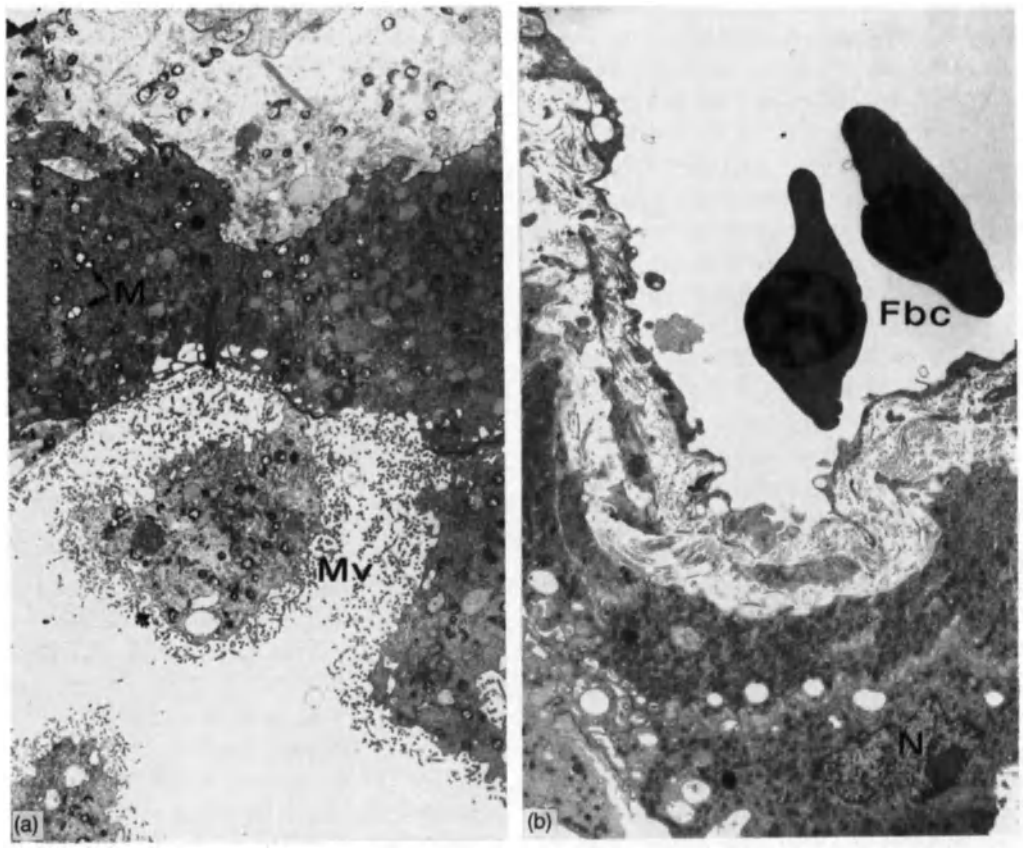


Figure 7.30 Ultrastructure of the full-term placenta of the tammar, *Macropus eugenii*, pulled free from the uterine epithelium. (a) Avascular, bilaminar yolk sac membrane, with numerous microvilli (Mv) on the ectodermal layer which would appose to the uterine epithelium. There are numerous mitochondria (M). Magnification ×1700. (b) Vascular, trilaminar yolk sac membrane in the region of the sinus terminalus shown with two fetal red blood cells (Fbc). Ectodermal cells have numerous enclosed granules and vesicles. Magnification ×2700.

centa. It thus appears that the yolk sac membrane, while capable of limited progesterone synthesis, has enzymes associated predominantly with steroid catabolism (Heap *et al.*, 1980). Although some parallels can be seen between steroid metabolism in the yolk sac placenta of *M. eugenii* and those of some eutherian mammals with superficial implantation, an important difference is that aromatase activity, which is pronounced in the placentae of pig and horse (see Gadsby *et al.*, 1980), is undetectable in *M. eugenii* (Heap *et al.*, 1980; Renfree *et al.*, 1984b), although it is detectable in the endometrium, brain, ovaries and adrenal tissues (Heap *et al.*, 1980; Callard *et al.*, 1982; Renfree *et al.*, 1984b) (Figure 7.31).

Two other observations indicate that the fetoplacental unit of macropodid marsupials may influence the maternal hormones systemically (Tyndale-Biscoe and Renfree, 1987). First, the lengths of gestation in two species of kangaroo, *Macropus giganteus* and *M. fuliginosus*, are 36 and 31 days but the gestation period of *M. giganteus* females mated to *M. fuliginosus* males is intermediate at 34 days (Kirsch and Poole, 1972; Poole, 1975), reflecting the hybrid genotype of the fetuses. The female progeny themselves had oestrous cycles intermediate in length between the parent species and, when backcrossed to *M. fuliginosus* males, had even shorter gestations (32 days, n = 12) (Poole, 1975). Second, Merchant's (1979) demonstration that gestation advances the time of oestrus by up to 3 days in *M. eugenii* and other species and that associated changes in progesterone, prolactin and luteinizing hormone are also advanced (Tyndale-Biscoe *et al.*, 1983) is strong evidence for a fetoplacental influence operating in the first instance on the corpus luteum and indirectly on the pituitary thereafter. A summary of the ways the placenta may interact with the endometrium and ovary is shown in Figure 7.32.

All these effects may be said to be a maternal recognition of pregnancy, and some or all may be due to endocrine signals from the placenta, although direct evidence for this has not yet been obtained. The vascular anatomy of the reproductive tract, which favours local circulation, would enable some of these to be unilateral effects (Towers *et al.*, 1986), so that high concentrations may not be necessary. It is of interest that laparotomy alone at the time of attachment of the yolk sac placenta (days 18 and 19) interferes with subsequent parturition in *Setonix brachyurus* and *M. eugenii*, which may further implicate the placenta as an important component in normal gestation (I.R. Young and Renfree, 1979). This form of maternal recognition of pregnancy is probably restricted to the Macropodidae, as recent studies on *D. virginiana* (Harder and Fleming, 1981) and *Dasyuroides byrnei* (Fletcher, 1983) disclosed no evidence for an endocrine recognition of pregnancy in either species.

7.8 ACQUISITION OF ENDOCRINE ORGANS DURING DEVELOPMENT

7.8.1 EMBRYONIC AND FETAL GROWTH

Up to the unilaminar blastocyst stage there is no increase in diameter of the embryo, but thereafter there is rapid expansion of the blastocyst through absorption of fluid into the blastocoele across the trophoblast. A feature of marsupial embryogenesis is the relatively slow rate of embryonic development, which only increases during the later stages of pregnancy (Tyndale-Biscoe and Renfree, 1987) (Figure 7.33).

There are few complete, timed studies of embryonic development in marsupials, and the first, of the opossum *Didelphis virginiana*, remains the most detailed today (McCrady, 1938), and provides a useful guide to the significant developmental features of marsupial embryos (Table 7.4; Figure 7.34).

There has been a burgeoning of studies over the last two decades on particular stages of embryonic development, particularly the pre-implantation stages (see Selwood, 1992)

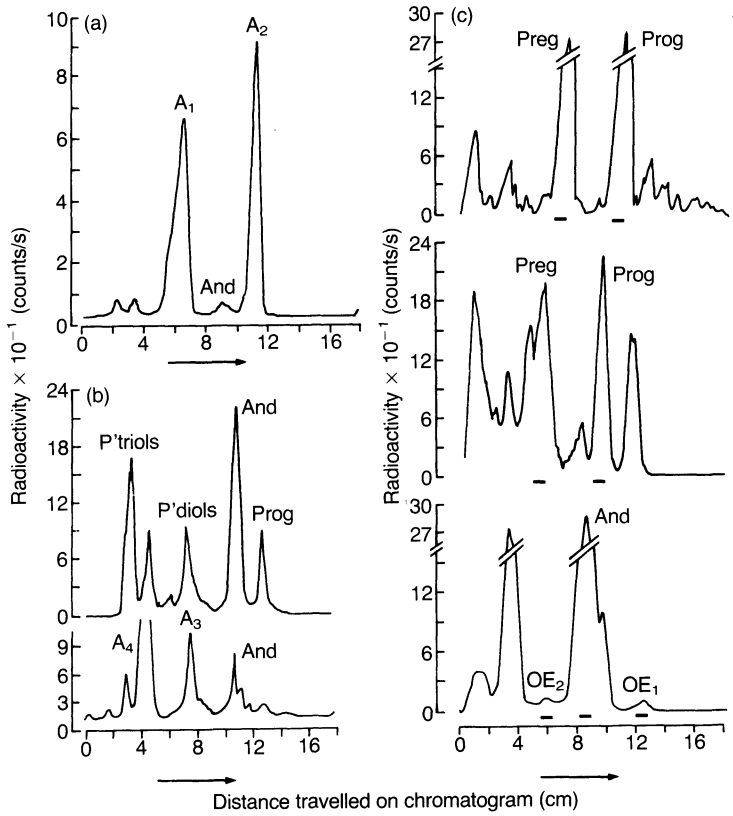


Figure 7.31 Steroid metabolism in fetal and maternal tissues of the tammar wallaby. (a) Yolk sac metabolism of [^{3}H]androstenedione *in vitro*. Tissue was obtained at day 20 after removal of pouch young and incubated for 3 h at 37°C with labelled androstenedione. After incubation, tissue and medium were extracted and the neutral steroid fraction was applied to a thin-layer chromatogram, which was developed in the solvent system, methylene dichloride–methanol (5:30, v/v). The diagram shows a radiochromatogram scan of radioactivity. A_1, 3β-hydroxyandrostan-17-one (androsterone); And, androstenedione (not metabolized); A_2, 5α-androstane-3,17-dione. (b) Endometrial metabolism of (a) [^{3}H]progesterone and (b) [^{3}H]androstenedione *in vitro* in the tammar wallaby. Tissue was obtained at day 15 and day 11, respectively, after removal of pouch young. Diagram shows radiochromatogram scan of radioactivity in the neutral steroid fraction after application to a thin-layer chromatogram plate which was developed in the solvent system, methylene dichloride–methanol (75:30, v/v). Prog, progesterone; And, androstenedione; P'diols, pregnanediols; A_3 and A_4, unknowns (see Table 7.4). (c) Radiochromatogram scans showing separation of [^{3}H]-labelled substrates and products by thin-layer chromatography. Top: Metabolism of [^{3}H]pregnenolone (Preg.) to progesterone (Prog.) by corpus luteum obtained on day 20 of pregnancy after removal of pouch young (RPY). Middle: Metabolism of [^{3}H]pregnenolone by remaining ovary (interstitial tissue + cortex) on day 15 of pregnancy after RPY; Bottom: Metabolism of [^{3}H]androstenedione (And.) by remaining ovary (interstitial tissue + cortex) on day 25 of pregnancy after RPY. OE_2, oestradiol-17β; OE_1, oestrone. Bars indicate chromatographic behaviour of corresponding authentic standards. [Redrawn from (a and b) Heap *et al.* (1980); (c) Renfree *et al.* (1984b).]

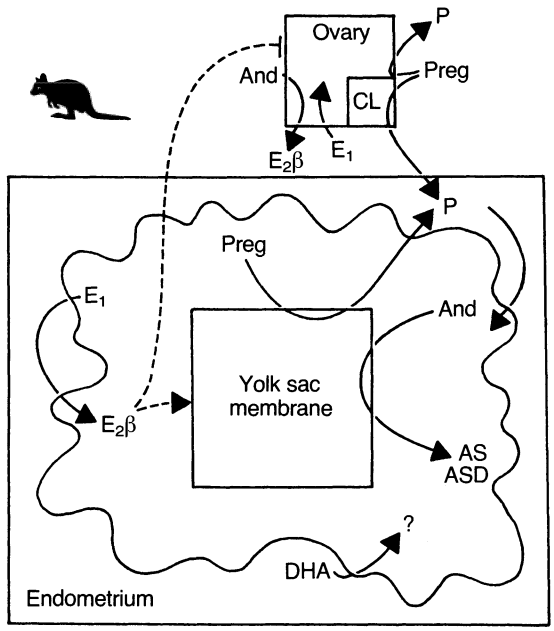


Figure 7.32 Suggested interactions of steroid hormones in the tammar *Macropus eugenii* during pregnancy after removal of pouch young, based on incubation *in vitro* with labelled steroids (see Figure 7.31). After incubation, although the placenta, weighing 100 mg, has only low levels of steroid synthetic activity, this may be sufficient to provide local stimulation of the endometrium. And, androstenedione; AS, androsterone; ASD, androstanedione; CL, corpus luteum; DHA, dehydroepiandrosterone; E_1, oestrone; $E_2\beta$, oestradiol-17β; Preg, pregnenolone; P, progesterone. Based on data in Heap *et al.* (1980) and Renfree *et al.* (1984b). [From Renfree (1980c), redrawn by Tyndale-Biscoe and Renfree (1987).]

and the fetal stages (Renfree, 1972a,b; Bancroft, 1973; Renfree and Tyndale-Biscoe, 1973, 1978; Shaw and Rose, 1979; Tyndale-Biscoe, 1979; Krause *et al.*, 1979a,b; Selwood, 1980; Walker and Rose, 1981; Hughes and Hall, 1984, 1988; Hall and Hughes, 1987; Krause and Cutts, 1983, 1984, 1985; Krause and Leeson, 1973; Hall, 1988), and most of these have been reviewed in detail by Tyndale-Biscoe and Renfree (1987); newer work on the development of the endocrine organs is given in the following section, so the following description is brief to provide the necessary framework for discussion.

After the formation of the primitive streak the embryo acquires a bilateral symmetry, with Hensen's node at the anterior end (Figure 7.35).

The first somites are seen in vesicles are between 4 and 8 mm in diameter. Amniogenesis occurs relatively late in marsupials, soon after the appearance of somites (Tyndale-Biscoe and Renfree, 1987). Cervical flexure occurs when the embryo has about 18–20 somites and the neural tube is closed along most of the length of the embryo (Figure 7.36).

It is about this time that the shell membrane (see Krause and Cutts, 1983), which has become more and more attenuated, ruptures, and a close and direct apposition of the trophoblast to the uterine epithelium becomes possible. At this time in the tammar there is a specific proteinase in the trophoblast endoderm complex for 1 day (day 18–19) exclusively in the bilaminar yolk sac (Denker and Tyndale-Biscoe, 1986). This proteinase activity is correlated with the dissolution of the shell membrane and the subsequent attachment of the trophoblast to the uterine epithelium in the bilaminar, but not trilaminar, region. Whereas all development up to this stage has been relatively slow and occupied two-thirds of the active gestation period, organogenesis is relatively rapid, and the time it takes is only a two-fold or so difference in very divergent species which may also have very different body sizes (Figure 7.33).

The placental membranes apposed to the uterine wall consist of vascular and non-vascular portions of the yolk sac in most marsupials (Sharman, 1961; Hughes, 1974, 1984) (Figure 7.30). Only a small area of true chorion remains and the allantois is enclosed in the fold of the yolk sac membrane, except in the Peramelidae, in which it forms an intimate attachment (Flynn, 1923; Padykula and Taylor, 1976, 1977, 1982). In the tammar, the

allantois never reaches the chorion, and as gestation proceeds it accumulates urea in the allantoic fluid, presumably via the patent Wolffian ducts draining the mesonephros (Renfree, 1973a). A similar arrangement of fetal membranes has been described for a variety of macropodids and phalangers (Tyndale-Biscoe and Renfree, 1987). As noted above, some marsupials have an allantoic placenta in addition to a yolk sac placenta, and the most elaborate form of allantoic placentation is found in the bandicoots – *Perameles nasuta*, *P. gunnii* and *Isoodon obesulus* – where a true chorioallantoic placenta is present in addition to the yolk sac placenta (Padykula and Taylor, 1977).

At birth, the tiny young is able to climb to the pouch unassisted by the mother (Cannon *et al.*, 1976), using its well-developed forelimbs (Hughes *et al.*, 1989; Renfree *et al.*, 1989). Its lungs are functional, the nostrils open and the olfactory centre of its brain is well developed (Gemmell and Rose, 1989a,b). The mouth, tongue and digestive system, including liver and pancreas, are sufficiently developed to cope with the change

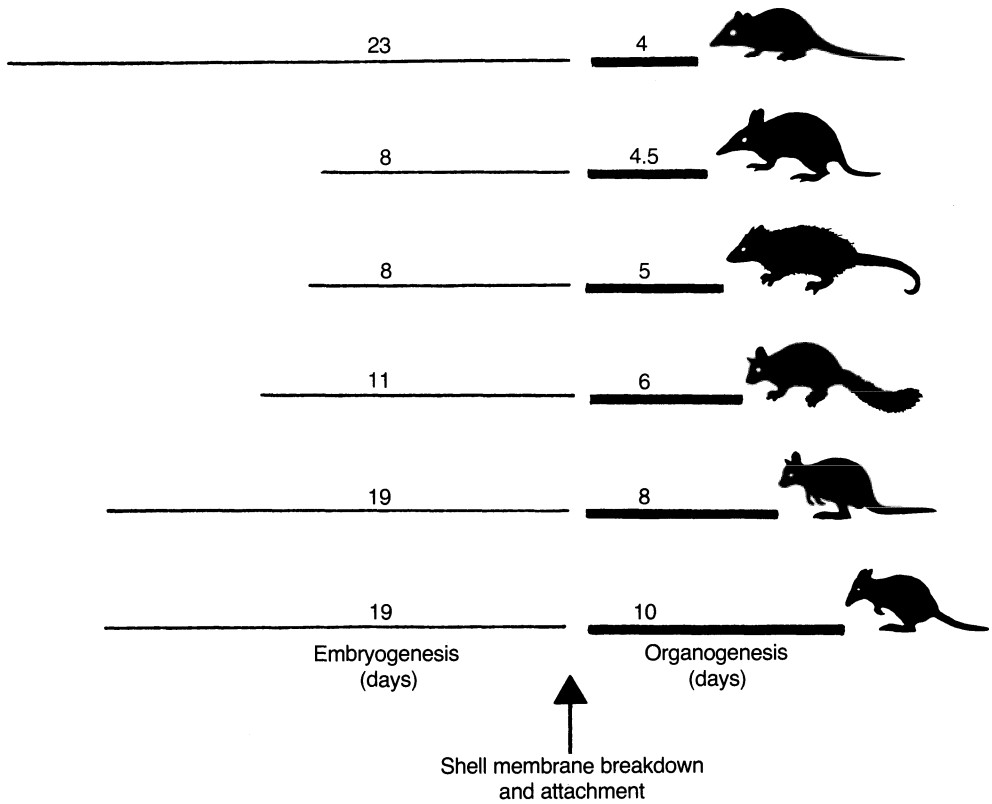


Figure 7.33 Relative duration (days) of the preattachment phases in six different marsupials. Species from top to bottom are *Antechinus stuartii*, *Perameles nasuta*, *Didelphis virginiana*, *Trichosurus vulpecula*, *Macropus eugenii* and *Potorous tridactylus*. Numbers given are days of gestation. The period of organogenesis is relatively constant (thick line) amongst these divergent species, which range in size from less than 50 g to over 6 kg. The preattachment phase when embryogenesis occurs is however very variable, and may be extended even further by embryonic diapause. (From Tyndale-Biscoe and Renfree, 1987, with permission.)

Table 7.4 Normal embryonic stages in *Didelphis virginiana*. (From McCrady, 1938)

Stage	*Developmental features*	*Day of pregnancy*	*Ovum or vesicle diameter (mm)*
1.	One-celled ovum (tubal). Fertilization 12–30 h p.c.	0	0.122×0.104
2.	Two-celled ovum (uterine). Cleavage complete 54 h p.c.	1	0.6
3.	Three-celled ovum	2	
4.	Early four-celled ovum with large blastomeres		
4'.	Late four-celled ovum with small blastomeres		
5.	Six-celled ovum		
6.	Eight-celled ovum		
7.	Twelve-celled ovum		
8.	Sixteen-celled ovum		
9.	Thirty-two-celled ovum		
10.	First endodermal mother cells	3	
11.	Earliest medullary plate and considerable yolk in gastrocele		0.20
12.	Expanded and attenuated vesicle with endodermal cells still almost entirely under medullary plate		0.34
13.	Ellipsoid blastocyst eccentric in albumen and with endodermal cells spreading toward equator	4	
14.	Spherical bilaminar blastocyst	5	0.75
15.	Clear spot in medullary plate	6	1.0
16.	First mesodermal cells forming cloud in the clear spot		1.4
17.	Primitive streak with mesodermal crescents		2.0
18.	Hensen's node	7	2.2
19.	Primitive groove. Mesoderm still only beneath medullary plate		
20.	Clear spot in front of Hensen's node. Mesoderm extending beyond medullary plate		3.2
21.	Notochord equal to or shorter than primitive groove		3.7
22.	Notochord longer than primitive groove. First somites forming		
23.	First coelomic rudiments. No heart. No optic cups		
24.	Optic cups but no contact of medullary folds	8	
25.	Contact of medullary folds, but no proamniotic fold		(GL* 5.5)
26.	Proamniotic fold, but no caudal amniotic fold		10.0
27.	Caudal amniotic fold, but anterior neuropore and otocysts still open	9	
28.	Anterior neuropore and otocysts closed. Amniopore still open		
29.	Amniopore closed. Forelimb becomes a club		
30.	Forelimb paddle. Secondary lumber flexure	10	
31.	Diameter of allantois approximately one-third length of body Hind limb bud. Frontal process		
32.	Diameter of allantois approximately two-thirds length of body Hind limb club.	11	
33.	Diameter of allantois equal length of body. Hind limb paddle Eyelid folds		
34.	Oral shield. Epitrichium covers eyes, ears, and sides of mouth Claws on forelimb digits	12	
35.	Oral shield practically resorbed. Umbilical cord lost or very much constricted	12½	
		BIRTH	

*GL, greatest length of embryo

to a milk diet (Janssens and Ternouth, 1987). By contrast, features such as the eyes, the hind limbs and the gonads remain undifferentiated (Hughes and Hall, 1988; O *et al.*, 1988); the scrotum is visible in embryos from day 22 RPY, but the pouch can be clearly seen only after about 8 days after birth (Renfree and Short, 1988); the metanephros is differentiated but not functional immediately (Wilkes and Janssens, 1988). Nevertheless, the entire period of differentiation is accomplished in a short time, which prepares the neonate for birth and for its more lengthy stay in the pouch whilst the rest of its growth

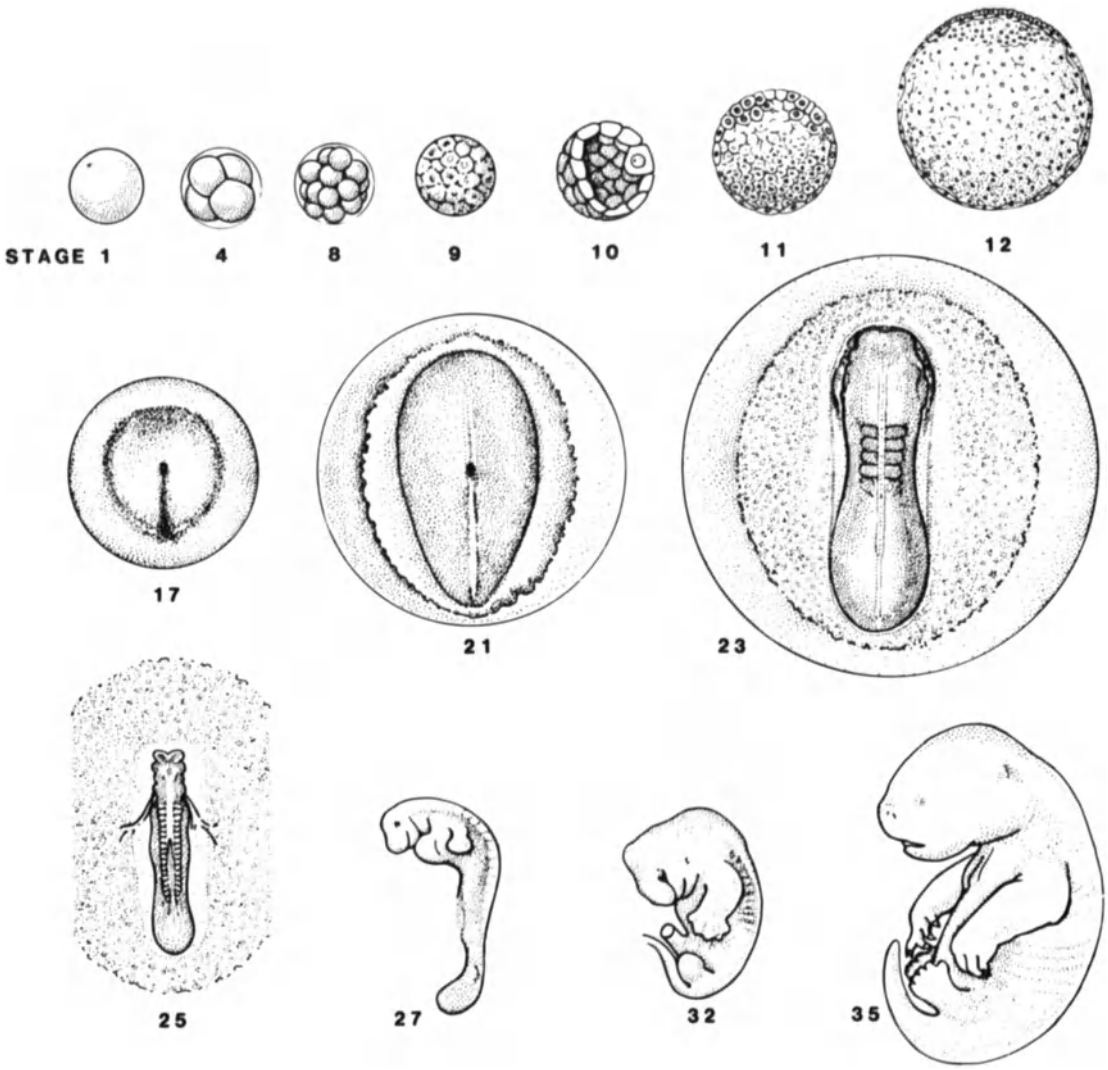


Figure 7.34 Representative stages of development of the American opossum, *Didelphis virginiana*, redrawn from McCrady (1938). Each horizontal row is drawn to the same scale. Thus stages 1–12, 17–23, and 25–35, are to the same scale. The diameter of the oocyte (stage 1) is about 0.11 mm and the diameter of the vesicle (stage 12) is 0.34 mm. No representation of the mucin layer or shell membrane is shown in this series. Detailed descriptions of the key developmental events are given in the accompanying table (Table 7.4).

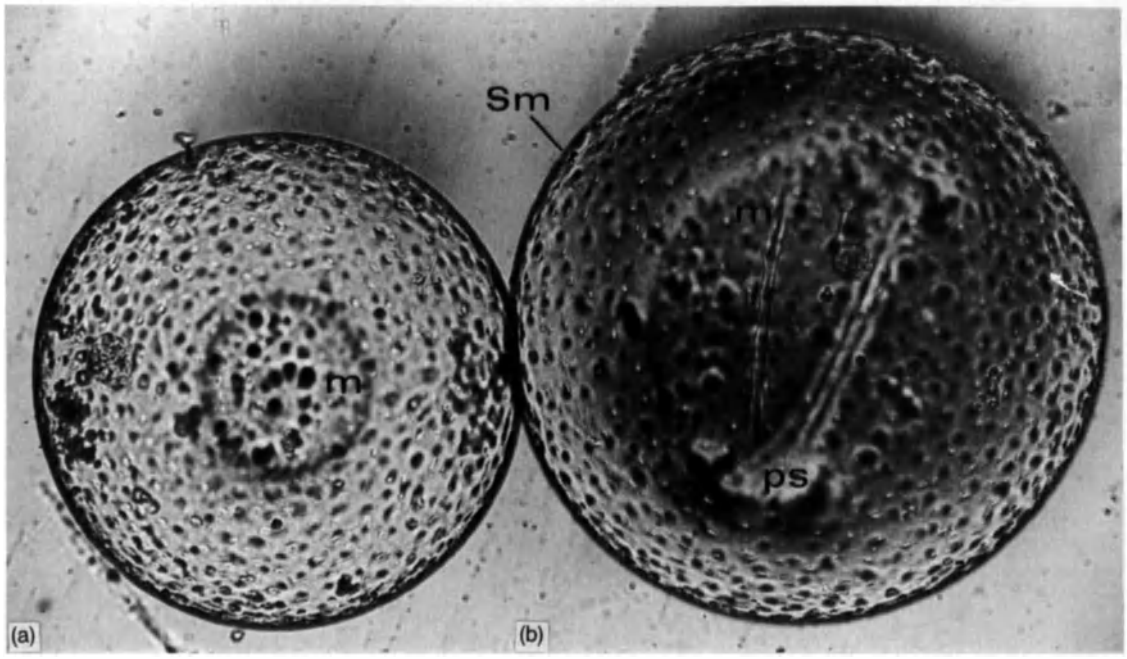


Figure 7.35 Developing embryos from one uterus of the quoll, *Dasyurus viverrinus*, 15 days post coitum. Vesicle (a) is 2.5 mm diameter, and shows the beginnings of the medullary plate (m), equivalent to McCrady stage 12 (see Figure 7.34). The trophoblastic nuclei are prominent and appear in dots. Vesicle (b) is 3.5 mm diameter, and is at the primitive streak (ps) stage, equivalent to McCrady stage 19 (see Figure 7.34 and Table 7.4). (Photograph kindly provided by Dr T.P. Fletcher.)

and development is completed (Sharman, 1973).

7.8.2 ENDOCRINOLOGY OF THE FETUS AND NEONATE

The fetal stages of all marsupials are of a very short duration, and given the range of adult body sizes, the duration of organogenesis is remarkably similar between species (Figure 7.33) whereas the period from fertilization to the end of embryogenesis is a very variable period ranging from a few days up to 11 months in those species which have embryonic diapause. By contrast, the period from birth to weaning is very long in marsupials, and maturation of many of the organ systems occurs during early pouch life.

Studies of the endocrinology of the fetus are virtually non-existent, but nevertheless data are accumulating on the morphology of the endocrine glands and the first appearance of hormones in late fetal and early pouch life. As will be seen in the following section, observations on endocrine responses in the mother which are dependent on the presence of a fetus are more numerous and support the idea that the fetus can certainly redirect maternal physiology by the influence of fetal hormones. This section will therefore concentrate on the few reports on the endocrine competence of the fetus and neonate.

The sequence of events during sexual differentiation in marsupials has recently been reviewed (Renfree *et al.*, 1987; Tyndale-Biscoe and Renfree, 1987; Renfree and Short, 1988; Short *et al.*, 1988; Sharman *et al.*, 1990; Shaw *et al.*, 1990; Renfree, 1992) so only the briefest summary will be given here. In the tammar wallaby, *Macropus eugenii*, the gonad is essen-

tially indifferent on the day of birth, and there are no morphological differences between males and females in their Wolffian and Mullerian duct systems, suggesting that the male gonad has not initiated the secretion of Mullerian inhibiting substance (MIS) from the future Sertoli cells, or androgens from the future Leydig cells (O *et al.*, 1988).

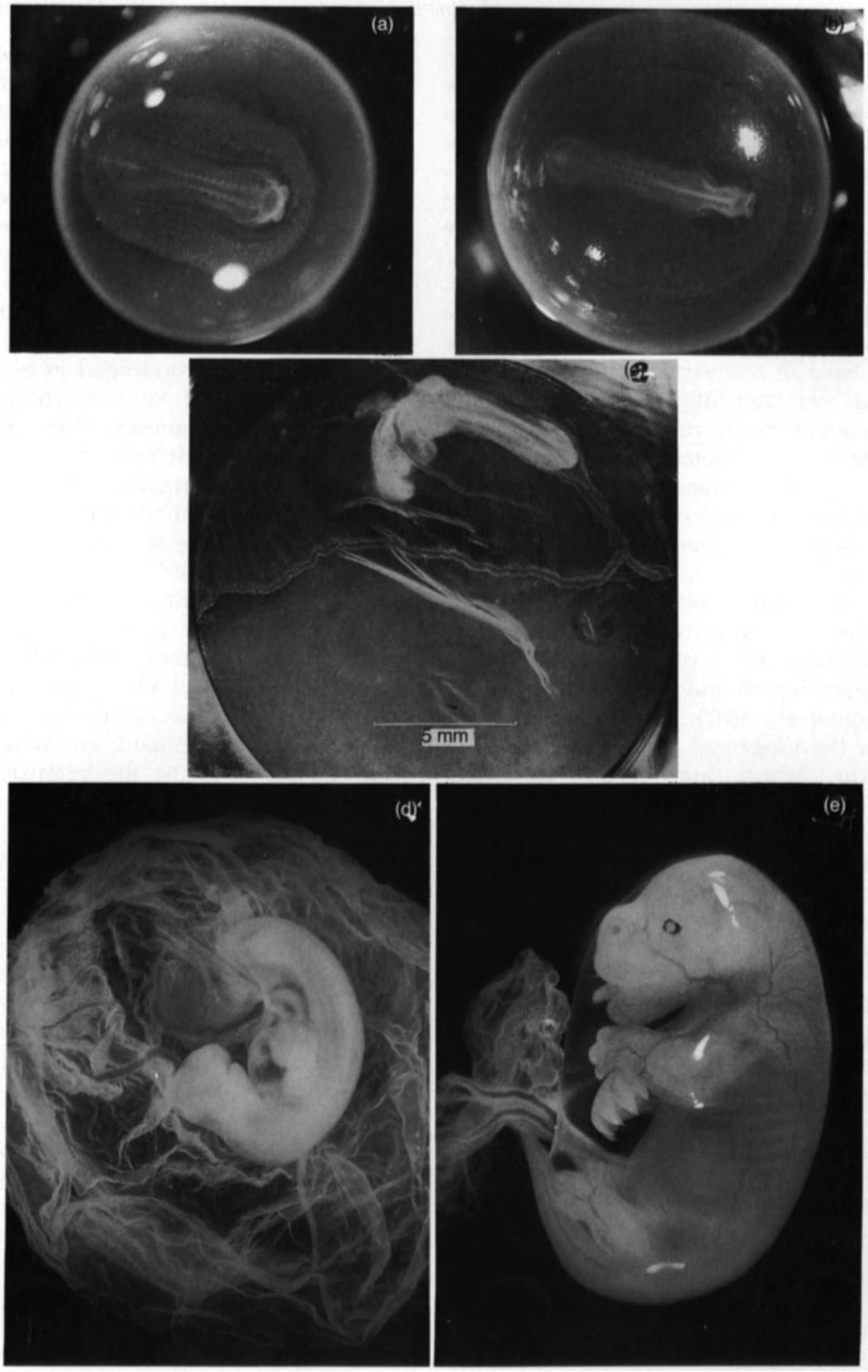
Hormonal secretion by the testis does, however, commence soon after birth. By the second day of pouch life the testes of tammars have developed seminiferous tubules and are secreting MIS (Hutson *et al.*, 1988). Testosterone production also appears to be initiated early in pouch life (Renfree *et al.*, 1992). In the tammar wallaby sufficient androgen is secreted in males before day 25 to prevent the regression of the Wolffian ducts that occurs in females (Shaw *et al.*, 1988). In another marsupial, the Virginia opossum, *Didelphis virginiana*, testes, but not ovaries, can convert pregnenolone to testosterone by day 19, and probably much earlier (George *et al.*, 1985). Likewise, the genital ducts, the urogenital sinus and the genital tubercle, which are known androgen-responsive tissues, contain the enzymes to convert testosterone to its active metabolite 5α-dihydrotestosterone.

In male marsupials the Mullerian ducts begin to regress by about day 10, apparently under the influence of MIS secreted by the testis (Renfree *et al.*, 1987; Hutson *et al.*, 1988; Short *et al.*, 1988; Shaw *et al.*, 1990). Treatment of neonatal marsupials with oestrogens prevents Mullerian duct regression, presumably by inhibiting MIS production or action (Burns, 1961; Shaw *et al.*, 1988). Wolffian duct development is controlled by androgens. Treatment of newborn female pouch young with testosterone prevents regression of the Wolffian duct, which normally disappears within a few weeks of birth (Burns, 1961; Shaw *et al.*, 1988).

By contrast, androgen is incapable of producing scrotal, processus vaginalis or gubernacular growth or suppressing mammary development in female neonatal tammars, and oestrogen is incapable of suppressing scrotal, processus vaginalis or gubernacular growth or stimulating mammary development in neonatal males (Shaw *et al.*, 1988). Likewise, Virginia opossums and neonatal grey opossums, *Monodelphis domestica*, treated with oestrogen retained their scrota (Burns, 1961; Fadem and Tesoriero, 1986). The scrotum has very low 5α-reductase activity, which is consistent with the idea that its differentiation is androgen independent (George *et al.*, 1985; Renfree *et al.*, 1992). Whilst the scrotum is present in male tammar fetuses from day 22 RPY, the pouch first becomes evident in genetic females on the eighth day after birth (Renfree and Short, 1988). A similar early scrotal differentiation occurs in *Trichosurus vulpecula* (Ullmann, 1993). The pouch does not seem to be under normal hormonal control, since androgen administration to female neonates will not inhibit pouch development, and oestrogen administration to male neonates will not induce pouch development in the tammar (Shaw *et al.*, 1988) or the grey opossum (Fadem and Tesoriero, 1986) or Virginia opossum (Burns, 1961).

Whilst the initial differentiation of the scrotum and mammary glands appears to be under direct genetic control (O *et al.*, 1988; Renfree and Short, 1988), and no adult male marsupial has mammary glands, there does seem to be a dichotomy between the Australian and American marsupials (Renfree, 1992). In the tammar, neither embryonic nor neonatal males ever have any sign of mammary primordia (Renfree and Short, 1988; Shaw *et al.*, 1990), but the didelphid opossum *Didelphis* and *Monodelphis* neonatal males have a reduced number of mammary anlage (Renfree *et al.*, 1990), which disappear after about 8 weeks of pouch life (Robinson *et al.*, 1991).

The pituitary is at an early stage of morphological development at birth in most marsupials. Presumptive somatotrophs,


(a)
(c)
5 mm
(e)

mammotrophs, thyrotrophs, gonadotrophs and corticotrophs have been identified in the pouch young of 1- to 50-day-old tammars by immunohistochemistry and electron microscopy (Leatherland and Renfree, 1982, 1983). The somatotrophs, together with non-granulated cells, make up 70% of the pars distalis. The mammotrophs, thyrotrophs and gonadotrophs increase in number and activity between 1 and 50 days post-partum, while the presumptive corticotroph cells are relatively most numerous in 25- to 30-day-old young, correlating well with the rapid increase in adrenal weight between days 20 and 40, the increase in cortical secretion (days 20–30) and the appearance of the adrenal medulla and adrenocortical zonatia (after day 20) (Catling and Vinson, 1976; Call *et al.*, 1980).

The structure of the red-necked wallaby (*M. rufogriseus*) pituitary at birth suggests that it, too, is functional, being well vascularized and many cells containing variable numbers of electron-dense membrane-bound granules (Walker and Gemmell, 1983b). The pituitary structure was similar in near-term fetuses and neonatal young (Walker and Rose, 1981; Walker and Gemmell, 1983b). However, no attempt was made to identify the cell types in this species.

There is some disagreement as to the cytological development of the pituitary in other species. In ultrastructural studies of the bandicoot *Isoodon macrourus*, Hall and Hughes (1985) could identify no obvious secretory granules in the homogeneous pituitary cells thought to be thyrotrophs before birth. Two days after birth presumptive somatotrophs were present, and 7 days after birth there were at least three cell types – presumptive thyrotrophs, presumptive gonadotrophs and presumptive somatotrophs. This pattern of differentiation is later than that reported for the tammar *M. eugenii* (Leatherland and Renfree, 1983), and Hall and Hughes (1987) concluded that the bandicoot pituitary may have little if any influence on the timing parturition after the brief 12½ days of pregnancy. In another non-macropodid, ultrastructural study on the brush-tailed possum *Trichosurus vulpecula*, the pituitary also appeared undifferentiated at birth and no secretory granules were present (Shorey, 1968). The first recognizable secretory cells were corticotrophs, which appeared at 16 days of pouch life, and acidophils (?somatotrophs) at 18 days (Shorey, 1968). These two studies differ from those of the recent study of Gemmell and Nelson (1988a) and Gemmell and Selwood (1993). These authors compared the ultrastructure of pouch young of the native cat, *Dasyurus hallucatus*, with that of *Trichosurus vulpecula* and *Isoodon macrourus* pouch young, and of the striped-faced dunnart, and found that there were three types of cells in the anterior pituitary containing different numbers of electron-dense, membrane-bound granules, and they identified them by comparison with the immunoreactive cell types described for the tammar by Leatherland and Renfree (1982, 1983). These were:

1. presumptive somatotrophs, mammatrophs and gonadotrophs;
2. presumptive corticotrophs; and
3. presumptive thyrotrophs.

Gemmell and Nelson (1988a) concluded that there are no differences in morphological differentiation between the pituitary cells of

Figure 7.36 Embryos of the tammar wallaby at (a and b) day 1 (diameter of vesicle 15 mm); (c) day 20; (d) day 21 (crown–rump length 8 mm) and (e) day 25½ (crown–rump length 16 mm) after reactivation of the diapause blastocyst. The yolk sac trophoblast forms the functional placenta, unlike the allantois, which never makes contact with the chorion. Birth occurs on day 26.4 after RPY. (From Renfree, 1982, with permission.)

newborn dasyurid, phalangerid and peramelids that they describe and those of the macropodids *M. eugenii* (Leatherland and Renfree, 1983), *M. rufogriseus* (Walker and Gemmell, 1983b), and *B. gaimardii* and *P. tridactylus* (Gemmell and Rose, 1989b) and that the pituitary of all species so far studied is functional at birth. Clearly, more studies need to be made using immunohistochemical techniques to identify the cell types and to resolve the differences in these observations.

Only one attempt has been made to measure pituitary hormones around the time of birth in marsupials, because of the generally small size of the late-term fetus and neonate. Wilkes (1984) reports that vasopressin is detectable in the pituitaries of 7-day-old tammar pouch young. The pituitaries contain about 10 ng of vasopressin (Wilkes and Janssens, 1986a,b), and are capable of releasing vasopressin from the pituitary in response to appropriate stimuli.

Adrenal function in the fetus and neonate is better described than that of the pituitary, especially in the macropodids *M. eugenii* and *M. rufogriseus*. In the tammar, the adrenal first appears at day 21 after RPY as a nodule of tissue lying close to the anterior pole of the mesonephric kidney, and has adrenocortical tissue from about day 22, with active 3βol-steroid dehydrogenase activity (Renfree, 1972b; Call *et al.*, 1980). Significant concentrations of cortisol (9–15 ng/ml) are present in fetal plasma from full-term fetuses at day 25 RPY, and the neonatal tammar adrenal can synthesize cortisol (Catling and Vinson, 1976). Similarly, the red-necked wallaby had 0.58 ng per adrenal in 12 adrenals from five neonates (day 27 RPY) and one fetus (day 26 RPY) (Walker and Gemmell, 1983b). In the bandicoot *I. macrourus* the cortisol concentration is 0.1 ng per adrenal ($n=9$) in the neonate (Gemmell *et al.*, 1982), but since the birth weight is less than that of macropodids (200–300 mg) the quantity is roughly proportional for the litter. Ultrastructurally the adrenal glands of newborn marsupials lack specific zones but consist of two distinct populations of cells (Gemmell and Nelson, 1988a). Dark staining granules are normally observed in catecholamine-secreting cells, and cells with large amounts of smooth endoplasmic reticulum and mitochondria are assumed to be the steroid-secreting cells and the source of the measured cortisol. The adrenal glands of the native cat and brush-tailed possum are similar in morphology to those of the bandicoot (Gemmell and Nelson, 1988a) red-necked wallaby (Walker and Gemmell, 1983b) stripe-faced dunnart (Gemmell and Selwood, 1993) and also of the tammar (Renfree, 1972b; Figure 7.37). Presumably all are capable of producing cortisol at birth.

The lung of the neonate also provides indirect evidence of adrenal function. The lung at birth is structurally immature but functional. Corticosteroids influence the development of the eutherian fetal lung by accelerating pulmonary maturation and increasing synthesis of surfactant. A similar maturation is observed in marsupials, and type II pneumocytes with their osmiophilic lamellar inclusions of surfactant appear at birth in *I. macrourus* (Gemmell and Little, 1982; Gemmell, 1986), *M. rufogriseus* (Walker and Gemmell, 1983b) *S. harrisii* (Hughes and Hall, 1988), *M. eugenii* (Randall *et al.*, 1984; Baudinette *et al.*, 1988; Hughes *et al.*, 1989; M.B. Renfree, unpublished observations), *D. hallacatus* and *T. vulpecula* (Gemmell and Nelson, 1988b) and *D. virginiana* (Krause and Leeson, 1973; Krause *et al.*, 1976). Thus, the evidence suggests that surfactant is produced in the structurally very immature lung of marsupials and that maturation of the surfactant system is a necessary prerequisite for breathing (Baudinette *et al.*, 1988). However, although a direct relationship between cortisol production and surfactant secretion remains to be shown, the general consensus of all these studies is that the fetal pituitary – adrenal axis of marsupials develops relatively early during the brief gestation to induce maturation of key organ systems (Table 7.5),

and is functional at birth, and, as in eutherians, is capable of influencing the length of gestation by initiating parturition.

7.9 CONTROL OF PARTURITION

The present evidence suggests that the control of parturition and the initiation of lactation are similar in marsupials and eutherians. Parturition requires the corpus luteum and both anterior and posterior pituitary, but, as in many eutherian mammals, in the macropodids the fetus and or placenta may also determine the time of birth. In marsupials the corpus luteum has an essential role for normal parturition at least up to the last few days of gestation (I.R. Young and Renfree, 1979; Bryant and Rose, 1986). Parturition occurs via the median vagina, and relaxin of luteal origin may be necessary for loosening the connective tissue of the vagina and urogenital sinus.

7.9.1 MYOMETRIAL ACTIVITY

Myometrial activity is quiescent during pregnancy, and as the gravid myometrium grows the concentration of collagen declines and the gravid myometrium, unlike the non-gravid myometrium becomes increasingly sensitive to oxytocin and prostaglandin (Shaw,

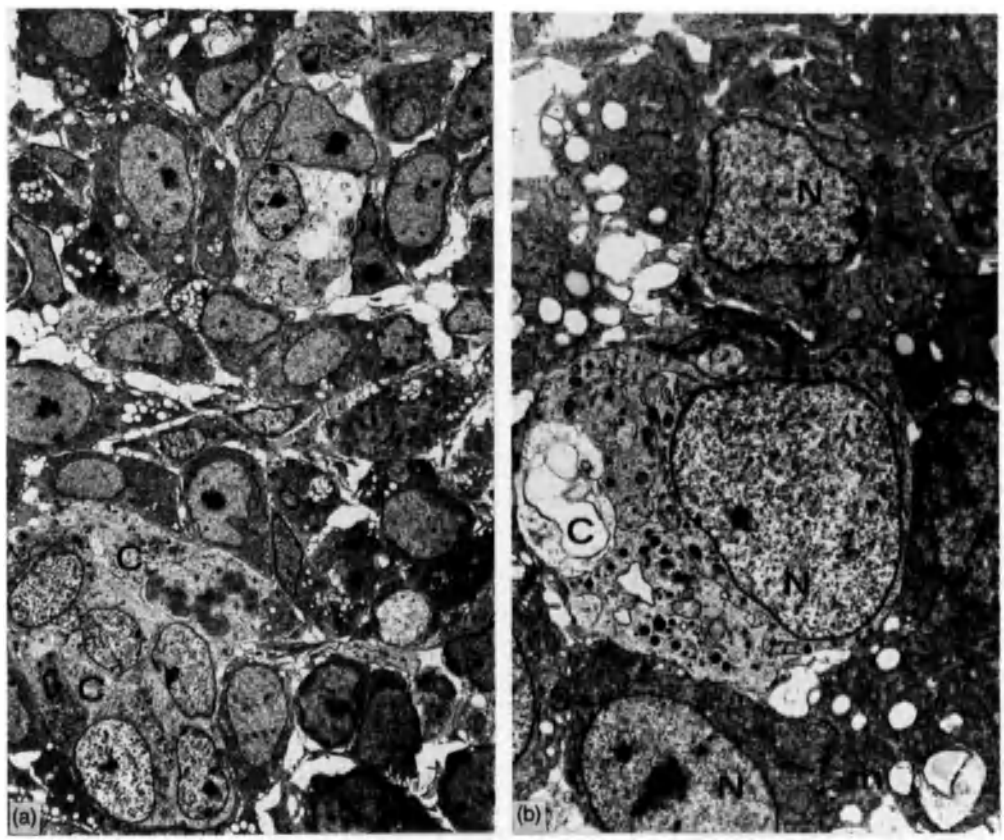


Figure 7.37 Transmission electron micrograph of the adrenal of a day 25 RPY tammar wallaby fetus. Two types of hormone-producing cells are seen: catecholamine-secreting cells (C) with distinct dark staining granules, and steroid (?cortisol)-secreting cells (S) with abundant endoplasmic reticulum and distinctive mitochondria (m). N, Nuclei. (a) Magnification ×1220. (b) Magnification ×3660.

Table 7.5 Time (days) during gestation or early pouch life in selected Australian marsupials when various organs appear functional

Species	*Total gestation length (days)*	*Duration of pouch life to weaning (days)*	*Pituitary*	*Adrenal*	*Lung*	*Gonad*	*Olfaction and taste*	*References*
M. eugenii	26.5	270	Birth	Day 22 RPY	Birth	2♂,10♀pp	Birth	Renfree (1972b); Catling and Vinson (1976); Call *et al.* (1980); Leatherland and Renfree (1982, 1983); Alcorn and Robinson (1983); O *et al.* (1988); Hutson *et al.* (1989); Renfree and Short (1988); Hughes *et al.* (1989)
M. rufogriseus	27	360	Birth	<day 26 RPY	Birth			Walker and Gemmell (1983b)
T. vulpecula	17.5	230	Birth	<Birth	Birth		Birth	Hughes and Hall (1984); Hall and Hughes (1987); Gemmell and Nelson (1988a–c); Gemmell *et al.* (1988)
D. hallucatus	?19	135–140	Birth	Birth	Birth		Birth	Gemmell and Nelson (1988a–d, 1989); Gemmell *et al.* (1988)
I. macrourus	12.5	60	Birth	<Birth	Birth	2♂,5♀pp	Birth	Ullman (1981a,b); Gemmell and Little (1982); Gemmell *et al.* (1982, 1988); Hughes and Hall (1984, 1988); Hall and Hughes (1985, 1987); Gemmell (1986); Gemmell and Nelson (1988a,b); Hall (1988)
S. harrisii	31	150–240			Birth		No taste buds Birth	Hughes (1982); Hughes and Hall (1984, 1988); Hall and Hughes (1985, 1987)

RPY, gestation days after removal of the pouch young; pp, days post partum; <indicates before.

1983a,b). Heller (1973) was the first to study contractility of the myometrium *in vitro*. In the quokka he found that isotonic contractile activity changed through the oestrous cycle, and that pregnancy influences uterine contractility. Early in the cycle (day 7 – day 18 RPY) uteri were more responsive to oxytocin than arginine vasopression, while late in the cycle (day 19 RPY to 2 days post-partum) the reverse occurred.

Myometrial activity *in vivo* has been recorded in the tammar by electromyography (I.R. Young, 1978; Renfree and Young, 1979). Spontaneous activity during seasonal or lactational quiescence is minimal. In non-pregnant females myometrial activity increases after about day 10 RPY, but activity remains low and cyclic in pregnant uteri at all stages of gestation. The gravid uterus has small responses to oxytocin at day 20 RPY, with increasing contractility and sensitivity in late pregnancy, but by day 1 post-partum both uteri are inactive and refractory (Shaw, 1983b; Figure 7.38).

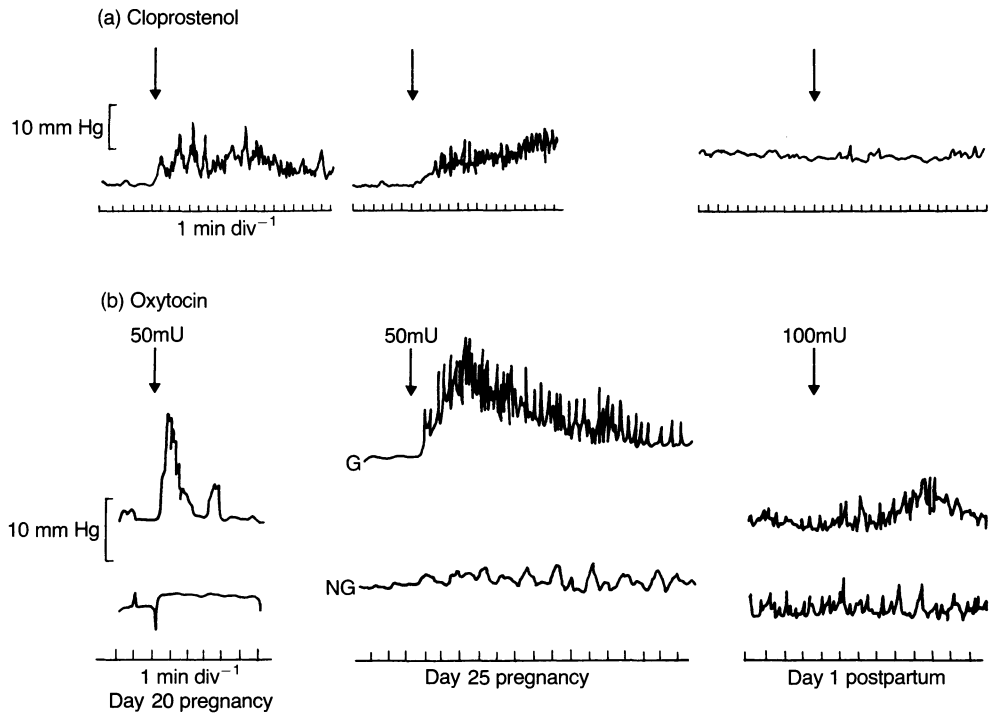


Figure 7.38 Myometrial activity in *Macropus eugenii*. (a) Effects of cloprostenol on intrauterine pressure during late pregnancy (day 20 and day 25) and 1 day post partum. Recordings were made from the gravid uterus or the recently evacuated uterus. Cloprostenol (0.5 μg) was injected into the lateral tail vein (arrow). The vertical scale is the same for all traces but the baseline differs. Data from Shaw (1983a). (b) Response of the myometrium of gravid (G) and non-gravid (NG) uteri to 50 mU or 100 mU oxytocin injection. At day 20, neither uterus showed much spontaneous activity, but the gravid uterus responded strongly to oxytocin both *in vivo* (shown here) and *in vitro*. At day 25, spontaneous activity in both uteri is slight. Oxytocin injections into the tail vein induced strong contractions in the gravid uterus but the non-gravid uterus responded weakly. In a female with a 1-day-old pouch young, spontaneous myometrial activity was slight. Oxytocin (100 mU) produced an increase in tonus of the post-partum uterus, but the stimulation was less pronounced than seen in prepartum uteri. Data from Shaw (1983b). (From Tyndale-Biscoe and Renfree, 1987, with permission.)

Since these effects differ between gravid and adjacent non-gravid uteri, they reflect a local rather than systemic influence. Myometrial responsiveness to the $PGF_{2\alpha}$ analogue cloprostenol increases markedly towards the end of gestation, but the myometrium is also refactory post-partum (Figure 7.38). Prostaglandin $F_{2\alpha}$ rises sharply at parturition (Lewis *et al.*, 1986; Fletcher *et al.*, 1990), and if administered near term will induce abortion (Renfree and Young, 1979; Shaw, 1983b). In a small number of tammars examined there is an increase in uterine $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ concentrations between day 25 and day 27, then a fall by 1 day post-partum (Shaw, 1983a). The extremely high concentrations measured in separated endometria and myometria at day 26, especially of PGE_2, show that at this stage the tissue can rapidly produce large amounts. The uterus is thus presumed to be the source of the circulating prostaglandins in the tammar.

The factors maintaining myometrial inactivity in gestation are not clear. In rabbits and sheep progesterone is an important inhibitor, but the success of pregnancy after lutectomy suggests that ovarian hormones are less important in this function in tammars and other marsupials. Placental progesterone production may maintain myometrial inactivity after lutectomy or ovariectomy, but the evidence is that placental progesterone production in tammars is very low. The inactivity of uteri during seasonal quiescence when peripheral progesterone concentrations are at their lowest also indicates that the myometrium does not need active inhibition by progesterone. Parturition is not invariably preceded by a fall in progesterone (Hinds and Tyndale-Biscoe, 1982a), and when progesterone levels are artifically elevated by injections or implants, parturition still occurs at the normal time (K.L. Ward and Renfree, 1984).

7.9.2 SOFTENING OF THE PSEUDOVAGINAL OR BIRTH CANAL

In marsupials the two separate uteri open into an anterior vaginal expansion from which the two lateral vaginae lead to the urogenital sinus. However, parturition occurs via the median vagina, which in all marsupials except the Macropodidae or in the honey possum, *Tarsipes*, is a short band of connective tissue that joins the anterior vaginal expansion with the urogenital sinus (Tyndale-Biscoe, 1966). After the first birth in macropodids and *Tarsipes* this birth canal becomes lined with squamous epithelium and remains patent, but in other marsupials the birth canal is rapidly repaired so that a few days after birth no evidence of the passage remains (Tyndale-Biscoe, 1966; Tyndale-Biscoe and Renfree, 1987).

A conspicuous feature of the fine structure of luteal cells of the four species of marsupial so far studied are small membrane-bound, electron-dense bodies of around 200 nm diameter (four-eyed opossum *Philander opossum*, Enders, 1973; brush-tailed possum *Trichosurus vulpecula*, Shorey and Hughes, 1973a,b; bandicoot *Isoodon macrourus*, Gemmell, 1979; tammar *Macropus eugenii* Tyndale-Biscoe, 1981). In the tammar and brush-tailed possum the maximum concentration coincides with the peak concentrations of bioassayable relaxin and progesterone, and they disappear rapidly after birth (Tyndale-Biscoe, 1966, 1981). Tyndale-Biscoe (1981) concludes that these granules may contain both relaxin and progesterone.

Although corpora lutea of tammars and brush-tailed possums contain high concentrations of bioassayable relaxin (Tyndale-Biscoe, 1966, 1981), no convincing effect of relaxin on softening the cervix and vaginal canal has been demonstrated in either species (Tyndale-Biscoe, 1966; Renfree and Young, 1979). In quokkas Lutrexin administration did ameliorate the effects of ovariectomy

(Tyndale-Biscoe, 1963a). However, the time of the ovariectomy (day 18–20) was late enough that some births would be expected at this time, as occurs in tammars and potoroos (I.R. Young and Renfree, 1979; Bryant and Rose, 1986). Progesterone treatment can also cause substantial loosening of the connective tissue of the urogenital strand in ovariectomized opossums (Risman, 1947), in the brush possum (Sharman, 1965a; Tyndale-Biscoe, 1966) and in the tammar (I.R. Young, 1978). It appears that both these hormones may be necessary for preparation of the birth canal.

Pregnancy goes to term but parturition fails in five species of marsupials so far studied without corpora lutea (after ovariectomy or lutectomy), as it does in tammars reactivated by exogenous progesterone for 10 days which have corpora lutea that remain inactive (Renfree and Tyndale-Biscoe, 1973). As in ovariectomized quokkas (Tyndale-Biscoe, 1963a) the fetuses were recovered dead in the vaginal cul de sac (Renfree and Tyndale-Biscoe, 1973), reinforcing the idea that the corpus luteum is essential for preparation of the genital tract, and the median vaginal canal in particular, for birth. However, these studies do not distinguish between the roles of the two main luteal hormones, progesterone and relaxin.

7.9.3 ENDOCRINE PROFILE AT PARTURITION

Progesterone is high prior to birth in all species so far studied, and is the major steroid hormone of the tammar (Renfree *et al.*, 1979; Hinds and Tyndale-Biscoe, 1982a; Shaw and Renfree, 1984; Lewis *et al.*, 1986; Fletcher *et al.*, 1990) and Bennett's wallaby (Walker and Gemmell, 1983a). Presumably, the need for the corpus luteum at parturition reflects a requirement for progesterone, yet progesterone withdrawal is not essential for parturition to occur (K.L. Ward and Renfree, 1984). Oestrogens are also elevated after parturition before the post-partum oestrus (Flint and Renfree, 1982; Shaw and Renfree, 1984; Harder *et al.*, 1984; Fletcher *et al.*, 1990), but oestrogen is clearly not essential in macropods for parturition, because oestrus does not invariably follow parturition, and in some species, such as the eastern grey kangaroo, *Macropus giganteus*, is not common, or does not occur post-partum at all, as in the western grey *M. fuliginosus*. In the swamp wallaby, *Wallabia bicolor*, oestrus is several days pre-partum (Tyndale-Biscoe, 1984). The Graafian follicle is the main source of oestradiol in the peripheral circulation in the tammar (Harder *et al.*, 1984, 1985) and presumably in the other species as well. Gonadotrophins are also not essential for parturition because animals immunized against GnRH give birth, although folliculogenesis is inhibited and there are no Graafian follicles (Short *et al.*, 1985).

The sequence of hormonal changes at parturition of the tammar is now clearly defined from recent detailed studies (Flint and Renfree, 1982; Shaw, 1983a; Tyndale-Biscoe *et al.*, 1983; Shaw and Renfree, 1984; K.L. Ward and Renfree, 1984; Harder *et al.*, 1984, 1985; Lewis *et al.*, 1986; Tyndale-Biscoe *et al.*, 1988; Fletcher *et al.*, 1990). Progesterone declines precipitously coincident with or occasionally within 6 h of birth. A sharp rise in oestradiol occurs 8 h after the progesterone drop, and oestrus 10 h later. The LH surge is dependent on the oestradiol rise and follows it by 7 h. Ovulation follows the LH surge by 24 h. Oestradiol levels are basal 24 h after the progesterone fall, and there is a transient small pulse of progesterone 16 h after oestrus and about 8 h after, which may reflect a switch from oestradiol secretion to progesterone by the granulosa cells (Figure 7.39).

In tammars sampled very frequently there is a marked peak of 800–1200 pg/ml of prostaglandin metabolite if collected within 10 min of birth, with values declining to less than 200 pg/ml within 45 min, and are nondetectable by 2 h post-partum (Lewis *et al.*, 1986; Fletcher *et al.*, 1990). Clearly, prosta-

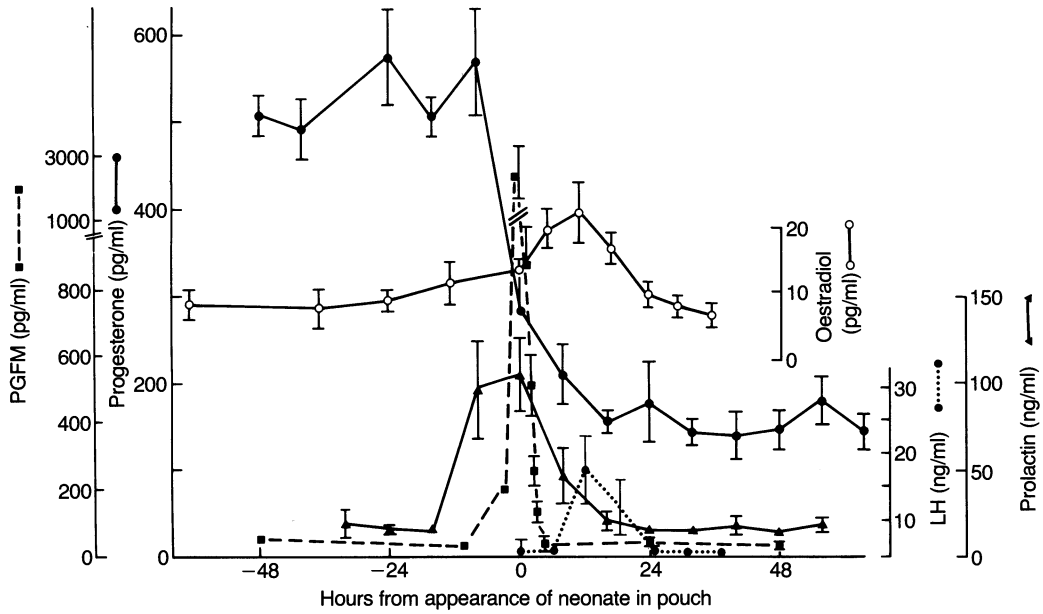


Figure 7.39 Concentrations of oestradiol-17β, progesterone, LH, prostaglandin $F_{2\alpha}$ metabolite (PGFM) and prolactin in peripheral plasma of tammars through the period of birth and post-partum oestrus. Data from Hinds and Tyndale-Biscoe (1982a,b); Tyndale-Biscoe *et al.* (1983); Shaw (1983a); Shaw and Renfree (1984); Harder *et al.* (1984, 1985); Lewis *et al.* (1986); Fletcher *et al.* (1990).

glandin is elevated during parturition, but the peak is very short-lived (Figure 7.39). In bandicoots, prostaglandin is elevated for longer periods (Gemmell *et al.*, 1980). In the red-necked wallaby, Walker and Gemmell (1983a) could not detect a marked rise, but if the pattern of prostaglandin release is like that of the tammar, a more frequent blood sampling regimen would be required to detect it.

In pregnant females there is a peak of prolactin at parturition which is not observed in non-pregnant females (Tyndale-Biscoe *et al.*, 1983), and it is now known that this pulse can, directly or indirectly, cause luteolysis (Tyndale-Biscoe *et al.*, 1988; Fletcher *et al.*, 1990; Hinds *et al.*, 1990b). This will be discussed in detail below.

7.9.4 FETAL ROLE IN PARTURITION

In all mammals maturation of the fetus is an important prerequisite for successful parturition, and in many eutherian species it is known that the fetus signals its readiness by influencing the maternal system. However, because of the similarity between the length of gestation and the oestrous cycle in marsupials, it was assumed that there was no fetal role in the timing and onset of parturition (Sharman, 1970). Since that time, a variety of studies indicate that macropodid marsupials do not differ from eutherians in this regard, and even the similarity of gestation length to oestrous cycle length has been brought into question by the finding of consistent differences which also depend on the presence of the fetus (Merchant, 1979; Merchant and Calaby, 1981). However, there is no evidence at present of any fetal influence on pregnancy in the opossum (Harder

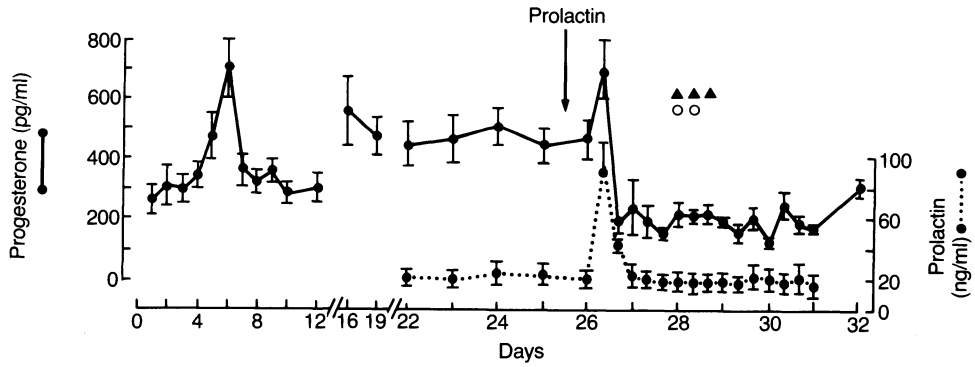


Figure 7.40 Effect of an exogenous pulse of prolactin on concentration (mean ± SEM) of progesterone (—) and prolactin (- - -), and on the time of oestrus (○) and the LH pulse (▲) in five non-pregnant tammars which were injected with 200 μg of prolactin on day 26. Values for progesterone to day 12 have been synchronized to the modal day of the early pulse of progesterone. (Redrawn from Tyndale-Biscoe *et al.*, 1988.)

and Fleming, 1981; Fleming and Harder, 1981b), or in any other non-macropodid so far studied (F. Stewart and Tyndale-Biscoe, 1983).

In the tammar the interval from oestrus to post-partum oestrus is shorter than the interval from oestrus to the next oestrus in non-pregnant tammars, an effect ascribed to the presence of the fetus. In pregnancy, the pulse in prolactin which is coincident with a sharp fall in progesterone around the time of parturition is associated with the presence of the fetus and not the stage of the ovarian cycle (Tyndale-Biscoe *et al.*, 1983; Hinds and Tyndale-Biscoe, 1982b, 1985), and it also is responsible for inducing luteolysis (Figure 7.40; Tyndale-Biscoe *et al.*, 1988; Hinds, 1990).

The short-lived rise in prostaglandin coincident with parturition (Lewis *et al.*, 1986) is now known to cause the release of prolactin, which in turn depresses the progesterone concentration (Hinds *et al.*, 1990b). However, if the prolactin pulse is blocked by the dopamine agonist bromocriptine, luteolysis still occurs after the prostaglandin pulse and parturition (Fletcher *et al.*, 1990). It therefore appears that either prolactin or prostaglandin will induce luteolysis after parturition. If the prostaglandin pulse is blocked by the prostaglandin synthetase inhibitor indomethacin, parturition fails though the prolactin pulse occurs (M.B. Renfree, G. Shaw and T.P. Fletcher, unpublished observation). There is also a short-lived pulse of prolactin just prior to birth and luteolysis in the Bennett's wallaby (Loudon *et al.*, 1990), but prostaglandin profiles at parturition have not yet been made after intensive blood sampling in this species.

Prostaglandin also has another unusual function in that it induces the adoption of the birth position by the female (Hinds *et al.*, 1990b; Shaw, 1990), an important component of birth behaviour in macropodids (see Renfree *et al.*, 1989). Even pre-pubertal females and males will adopt the birth position within 5 min of prostaglandin $F_{2\alpha}$ injection (Shaw, 1990), but the precise mechanisms involved are not yet defined. Prostaglandin also induces birth behaviour in five other marsupials so far tested: *Bettongia gaimardi, Isoodon obesulus, Petaurus breviceps, Trichosurus vulpecula* and *Thylogale billiardiei* (R. Rose, A.S. Macfayden and G. Shaw, personal communication). The question which now must be raised is how the fetus might induce the prostaglandin release.

Production of steroids by the fetal adrenal

is a common step in the early stages of initiation of parturition in many eutherian species, and typically begins several days before birth. In the sheep fetal cortisol induces an increase in synthesis of placental oestrogen, which in turn induces uterine prostaglandin release and uterine contractions (Liggins, 1982). What is the evidence for a similar cascade of events in marsupials?

In the tammar, presumptive adrenal tissue is evident by day 21 and the cortex is differentiated by day 22 RPY (Renfree, 1972b; Call *et al.*, 1980), and corticosteroids can be identified in the plasma of day 24 fetus (Catling and Vinson, 1976) (see above for more details). In a small group of tammars injected at day 20 or 22 with the 11 β-steroid dehydrogenase inhibitor metapyrone, three out of five delayed parturition (Renfree and Young, 1979), a result consistent with the idea that fetal cortisol might play an important role in the timing of parturition. We have recently shown that administration of dexamethasone at day 25 RPY results in parturition within 12 h, a day earlier than in control animals (Shaw *et al.*, 1992), providing the first direct evidence for a role of cortisol. However, whether there is any causal relationship between the fetal capacity to produce cortisol and the release of maternal prostaglandins remains to be determined. Since cortisol is also known to have many functions in the maturation of the eutherian fetus (e.g. lung maturation, surfactant release, lung liquid resorption, glucose storage, lactogenesis and closure of the ductus arteriosus) as well as parturition (Liggins, 1982), it is hardly surprising to find that the adrenal is functional by the time of birth in marsupials, too.

7.9.5 PROPOSED CONTROL MECHANISMS

Although any scheme for the control of parturition in marsupials, and in the tammar in particular, must still be regarded as hypothetical, it is now possible to update the model proposed by Renfree and Young (1979) for the tammar.

For normal parturition to occur, the corpus luteum needs to be present at least to day 23 (I.R. Young and Renfree, 1979). At this time, progesterone is high, and lutectomy at day 18 or day 23 causes an immediate fall in progesterone (Findlay *et al.*, 1983; Harder *et al.*, 1985) but not premature parturition. Relaxin is high at day 22, but has decreased to background levels by the day of birth. If relaxin and progesterone are necessary for softening the connective tissue of the cervix and the median vaginal canal, they have had sufficient effect by day 23 to allow birth. There is no evidence for oxytocin production from the corpus luteum (Curlewis *et al.*, 1988), so the maternal pituitary is the presumed source of any oxytocin. The pituitary gland is essential for parturition (Hearn, 1974) as evidenced by hypophysectomy, but gonadotrophins are not (Short *et al.*, 1985). Likewise, oestradiol from the developing Graafian follicle is also unimportant because those animals treated with anti-gonadotrophin releasing hormone had no follicular development but had normal corpora lutea and gave birth (Short *et al.*, 1985). This points to the need for a hormone(s) from the remainder of the anterior or posterior pituitary being essential for birth, and the two most likely candidates are prolactin from the anterior lobe and oxytocin from the posterior.

Prolactin, as described above, is released shortly before parturition in response to prostaglandin. Prolactin is luteolytic (Hinds *et al.*, 1990b), but since prolactin is not essential for luteolysis (Fletcher *et al.*, 1990) nor luteolysis for parturition (K.L. Ward and Renfree, 1984) and injections of prolactin at day 23 of gestation do not induce premature birth (Tyndale-Biscoe *et al.*, 1988), the peri-partum release of prolactin may be incidental to the process of parturition.

Oxytocin, like prostaglandin, can promote delivery of the young, via its action on smooth muscle contraction, but in most

species (except the rabbit) it has only a minor role and this is limited to the expulsive phase of parturition. In the rabbit it may act both directly on the uterus and to stimulate the release of $PGF_{2\alpha}$ from the endometrium. The role of oxytocin from the maternal pituitary in marsupial parturition has not been extensively tested as yet, but myometrial activity *in vivo* and *in vitro* can be stimulated by oxytocin, and the gravid uterus responds strongly (Shaw, 1983b; Tyndale-Biscoe and Renfree, 1987). Injections of oxytocin or prostaglandin cause massive contractions and expulsion of the fetus with considerable damage to it. It may be that oxytocin is necessary for evacuation from the uterus because in animals with a partial hypophysectomy in which the neurohypophysis was left intact the fetuses passed through the cervix but remained trapped in the vaginal canals (Hearn, 1973, 1974). We have recently delayed parturition using an oxytocin-inhibitor (M.B. Renfree, L.J. Parry and G. Shaw, unpublished results). It would be interesting if the oxytocin release induced the prolactin pulse, as has been recently shown in the rat (Mori *et al.*, 1990).

Prostaglandin has been measured in the uterus (Shaw, 1983b), but it is not yet established what causes the parturient prostaglandin release. Myometrial activity is increased after injection of prostaglandin (cloprostenol) during later pregnancy, but is refractory to it after parturition (Renfree and Young, 1979; Shaw, 1983a). It seems unlikely that a change in placental oestrogen induces prostaglandin release, because placental steroidogenesis occurs at a very low level (Heap *et al.*, 1980) and the placenta itself consists of only two or three cell layers (wet weight 100 mg at term). However, since there are large quantities of PGE_2 in the endometrium and myometrium (Shaw, 1983b), it is possible that this might induce the fetal adrenal to synthesize and release cortisol as it does in the sheep (Liggins, 1982; Thorburn *et al.*, 1988). It is also possible, though less likely, that fetal cortisol induces some change in the progesterone–oestradiol ratio, perhaps within the endometrium itself, since this tissue can convert oestrone to oestradiol (Heap *et al.*, 1980), but a direct effect on uterine prostaglandins cannot be dismissed at this stage. Tammar placentae also have 'gonadotrophic activity' but this needs to be further characterized (B. Hobson, L. Wide and M.B. Renfree, unpublished results).

In summary, it appears that the control of parturition has some features in common with the mechanisms of control of eutherians, but in the one species about which sufficient is known, the tammar, there is still a need for much more information. A hypothetical scheme (Figure 7.41) consistent with the present evidence summarizes the information presented above.

7.10 LACTATION: LACTOGENESIS AND GALACTOPOIESIS

7.10.1 INTRODUCTION

Lactation in marsupials lasts for relatively longer than in most eutherian species, to support the young, which are more immature at birth (Tyndale-Biscoe *et al.*, 1984; Findlay and Renfree, 1984). Although the cellular processes of embryological and prepubertal development, and the proliferation and differentiation of the mammary glands during pregnancy, are similar in all mammals, the temporal course of these events is different in marsupials (F.Stewart and Tyndale-Biscoe, 1983). The newborn marsupial climbs, unaided by the mother, into the pouch (Figure 7.42), where it spends a considerable period sustained by the specialized milk which changes in composition as the young grows (B. Green, 1984).

Pouch young remain permanently attached for about the first half of the period spent in the pouch, and are totally dependent on milk until weaning. At birth, young macropodids weigh only 0.5–1 g and are naked and blind,

depending on species, whereas at weaning they weigh up to several kilograms, are fully furred and fully mobile (Tyndale-Biscoe and Renfree, 1987). Again, most detailed information is at hand for the tammar (Tyndale-Biscoe and Janssens, 1988), and the maturation of the organs of the pouch young is completed by weaning, except in males whose testes mature at about 20 months when puberty occurs (Williamson *et al.* 1990) (Table 7.6).

The first appearance of fur is at about 150 days, when the young has some thermoregulatory ability, but homeothermy and full pelage are not established until days 180 and 200 days respectively. First excursions from

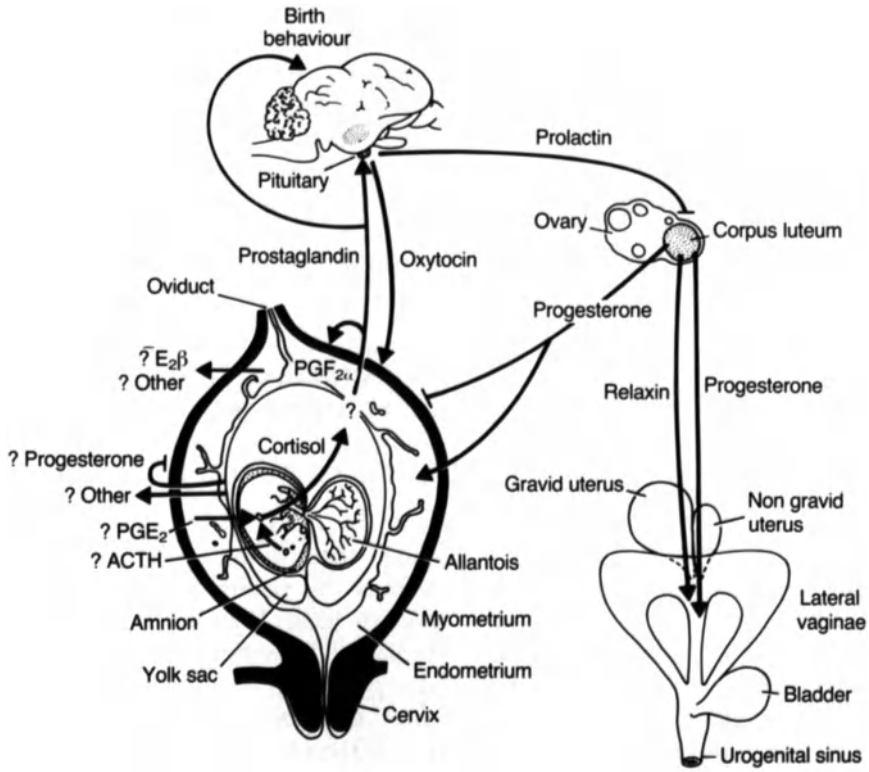


Figure 7.41 An hypothetical scheme consistent with present evidence to describe the possible control of parturition in the tammar wallaby. During pregnancy progesterone stimulates uterine secretory activity, and maintains myometrial quiescence. Fetal cortisol or endometrial oestrogen may stimulate prostaglandin synthesis in the endometrium. Fetal cortisol in turn could be stimulated by PGE_2, known to be present in endometrial tissue at this time, or by ACTH from the maturing fetal pituitary. Prostaglandin causes myometrial contraction in an oestrogen-sensitized uterus, but progesterone inhibits this effect. Prostaglandin also controls maternal behaviour and induces the adoption of the 'birth position'. Placental progesterone production may contribute to the observed unilateral effects of pregnancy. Progesterone and relaxin appear to be necessary for the softening of the cervix and the median vaginal canal to allow passage of the fetus to the outside. Prolactin during quiescence inhibits the corpus luteum, but at parturition it is released by prostaglandin to cause luteolysis. Oxytocin released from the maternal pituitary could stimulate myometrial contraction in concert with prostaglandin to evacuate the fetus through the cervix and then the long median vagina. (See text for further details and references.) ACTH, adrenocorticotrophic hormone; $E_2\beta$, oestrogens; $PGF_{2\alpha}$ and PGE_2, prostaglandins $F_{2\alpha}$ and E_2. Arrows show proposed stimulatory actions; T shows proposed inhibitory actions.

the pouch take place at 190 days, but the young is not out of the pouch permanently until over 250 days of age. Suckling may continue until the young is 340 days post-partum.

The number of teats generally limits the number of young that can survive in poly-ovular species like the family Dasyuridae, but many species have 'surplus' nipples. In all Macropodidae and Phalangeridae there are four teats available for the single-pouch young to choose for attachment. Extensive reviews on marsupial milk composition, mammary gland structure and the physiology of lactation are provided by B. Green (1984), Tyndale-Biscoe *et al.* (1984), Findlay and Renfree (1984), Tyndale-Biscoe and Renfree (1987), B. Green and Merchant (1988), Hinds (1988) and Nicholas (1988a), and on mother – young behavioural interactions by Russell (1982a,b, 1989).

7.10.2 MAMMOGENESIS AND LACTOGENESIS

Three broad stages can be recognized in marsupial lactation: the first (phase 1) of preparation of the mammary gland during pregnancy, (equivalent to lactogenesis stage I of eutherians; Fleet *et al.*, 1975; Cowie *et al.*, 1980), the second (phase 2) of initiation and maintenance of lactation during the first dependent period of pouch life, and the third (phase 3) when maintenance of an increased

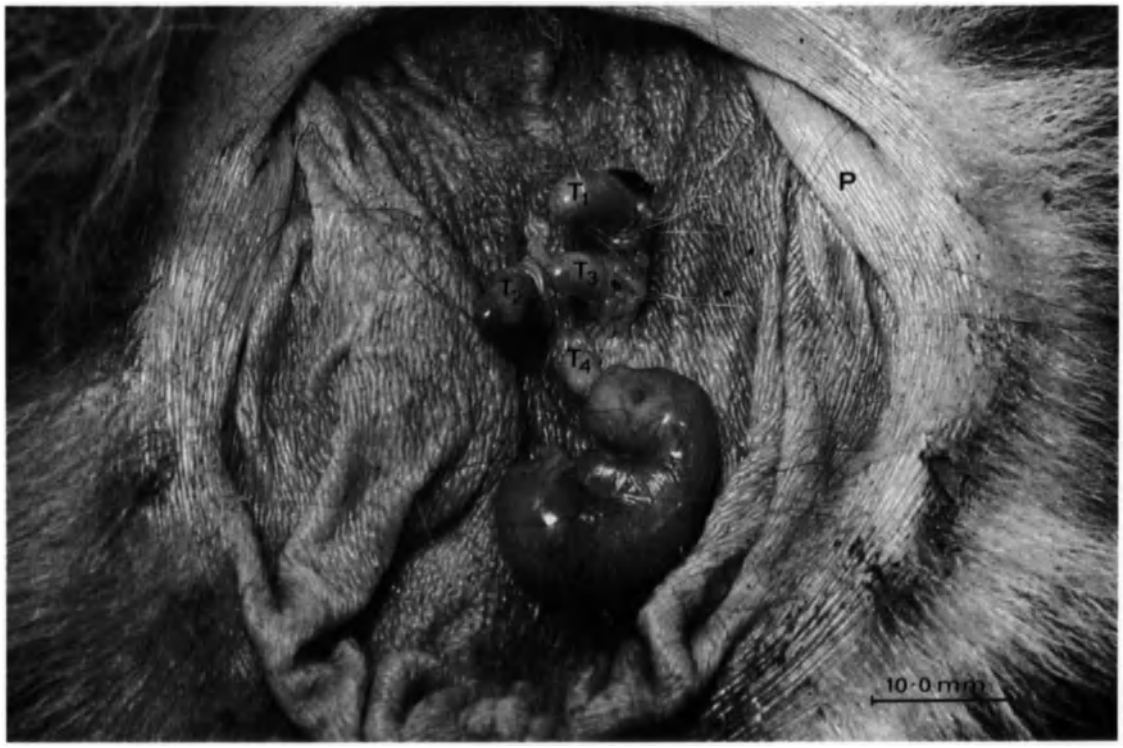


Figure 7.42 Neonatal tammar wallaby (24 h old) firmly attached to one of the four available teats (right posterior) (T_4) within the pouch (P). The right anterior teat (T_2) with dark tip supported the young of the previous lactation, while the left posterior teat (T_3) has never been sucked. The pouch is normally tightly closed by the pouch sphincter, but has been opened for photography. (Photo courtesy David Parer, PO Box 337, Mt. Eliza, Victoria, 3930, Australia.)

Table 7.6 Post-natal maturation of the tammar wallaby, *Macropus eugenii*. Data compiled from various authors[a]

Days from birth	*Developmental events*
0	Birth weight around 400 mg
	Scrotum visible externally
	Adrenocortical function
	Mesonephros functional kidney
	Neocortex of brain equivalent to 25-day sheep fetus or 12-day rat fetus
2	Seminiferous tubules in testis
4	First axons from retinal ganglion cell layer cross to opposite side of brain via optic chiasm
5–6	Initial appearance of cortical plate
10	Pouch young capable of gluconeogenesis
	Pouch externally visible in females
	Pituitary capable of synthesizing sufficient vasopressin for urine concentration
10–15	Cortical plate complete throughout the telencephalon
20	Carbonic anhydrase activity of red blood cells rises to adult levels
25	Metanephros completely replaces mesonephros
30	Thyroxine (T_4) is at adult concentrations, while triiodothyronine (T_3) is much lower
70	Mature pattern of eye innervation appears
	Completion of six layers of adult cerebral cortex
100	Voluntarily releases teat
120	Four- to fivefold increase in T_4 concentration
126	Milk only in forestomach
140	Eyes open, underfur starts to appear
150	T_3 levels increase
160	Head first out of pouch, able to stand
168	Milk only in forestomach
170	Starts to nibble grass
180	Homeothermy, thyroid function complete; T_4 levels decline to adult
190	First out of pouch
192	Able to hop
196	Milk and fragments of herbage in forestomach
196–245	Change in gluconeogenic regulation
196–252	Weaning. Doubling in pituitary vasopressin content
200	Full pelage
210	Milk and some herbage in forestomach
224	Herbage and some milk in forestomach
238	Ammonia content of urine falls to adult levels of about 18 mM, concentration of urea in urine approaches adult level of 550 mM. Herbage and a little milk in forestomach
250	Out of pouch permanently
252	pH of urine rises and approaches adult levels of pH 8
315–350	Fully independent: weight 1.6–2.5 kg.

[a] These data are derived from the chapters in *The Developing Marsupial*, edited by Tyndale-Biscoe and Janssens (1988b).

milk production occurs during the later, rapid growth stages before weaning. Phase 3 is equivalent to lactogenesis stage II of eutherians (Tyndale-Biscoe *et al.*, 1984; Findlay and Renfree, 1984; Tyndale-Biscoe and Renfree, 1987). The second stage of lactation in marsupials (phase 2) has no equivalent in eutherian lactation, and is characterized by low milk production and slow growth of the young whilst it remains permanently attached to the teat (B. Green, 1984; Tyndale-Biscoe *et al.*, 1984; Findlay and Renfree, 1984).

In eutherian mammals, significant changes in the mammary glands occur during pregnancy, but in marsupials the changes are subtler, and there is no difference in the changes between the oestrous cycle and pregnancy. O'Donoghue (1911) was the first to show the relation between enlargement and regression of the mammary glands of the quoll (*D. viverrinus*) with the development and regression of the corpus luteum. In this and in other marsupials, the glands reached their maximum size and degree of lobuloalveolar growth at the stage of the oestrous cycle at which parturition occurs in pregnant animals, and a small volume of clear colostrum can be expressed from each teat (Tyndale-Biscoe and Renfree, 1987). Pregnancy is not even necessary for successful lactation in marsupials, as has been amply confirmed by fostering neonates to mammary glands of virgin females at the equivalent stage of oestrous cycle to the peri-partum (Sharman, 1962; Sharman and Calaby, 1964; Merchant and Sharman, 1966; Clark, 1968; Findlay, 1982a,b; Tyndale-Biscoe *et al.*, 1984; Findlay and Renfree, 1984). After parturition or after transfer, only those glands to which the young became attached enlarge and lactate, while the other glands regress to the anoestrous size (Tyndale-Biscoe and Renfree, 1987).

Lutectomy, which reduces progesterone concentrations immediately (Findlay *et al.*, 1983; Harder *et al.*, 1984; Fletcher and Renfree, 1988; Figure 7.24), affects the ability of the mammary gland to secrete milk, depending on the stage of pregnancy when the corpus luteum is removed. In *M. eugenii* if the corpus luteum is removed at day 17 or day 21 RPY, the neonates fail to survive, but at day 23 or day 25 lactation is successfully established (I.R. Young and Renfree, 1979). Lutectomy at day 17 significantly reduces the mammary gland weights at parturition, but not if the operation is performed later at day 23 (Tyndale-Biscoe *et al.*, 1984; Hinds, 1988). As well as being necessary for lobuloalveolar growth of the mammary gland the corpus luteum, but not pregnancy, stimulates prolactin-specific receptors in mammary tissue of the tammar (F. Stewart, 1984). There is also a four- to five-fold increase in the size and weight of the mammary gland during pregnancy or the cycle (Findlay, 1982a; F.Stewart, 1984) (Figure 7.43), and this parallels the increase in plasma progesterone in the second half of the oestrous cycle (Hinds and Tyndale-Biscoe, 1982a).

A similar requirement for luteal secretions to prepare the mammary gland has been recorded in the opossum (Hartman, 1923), brush-tailed possum (Sharman, 1962) and the red kangaroo (Sharman and Calaby, 1964).

Although the corpus luteum is necessary for mammary gland growth and prolactin receptor synthesis, neither gonadotrophins nor oestradiol of follicular origin are necessary throughout pregnancy for lactogenesis (Short *et al.*, 1985). Mammary gland growth will occur during pregnancy even after hypophysectomy (Hinds, 1988), although the pituitary is essential to maintain lactation (Hearn, 1973, 1974).

In several eutherian mammals, progesterone withdrawal at parturition is a lactogenic trigger, but this is not the case in marsupials. Maintenance of high concentrations of progesterone through the peripartum period does not prevent the normal rise in lactose in mammary tissue which occurs after parturition, nor does lutectomy induce an early rise in lactose (Findlay *et al.*, 1983). Similarly, the

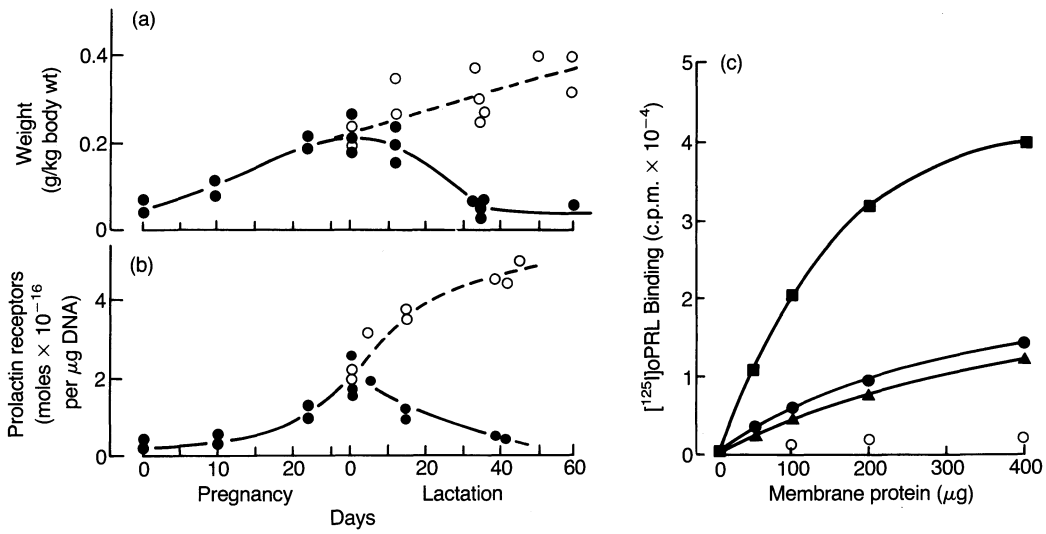


Figure 7.43 Changes in hormone levels and mammary glands throughout pregnancy and early lactation in the tammar *M. eugenii*. (a) Weight of the mammary glands and (b) prolactin receptor concentrations during the first 60 days of lactation in (○) sucked and (●) non-sucked glands. [Redrawn from Tyndale-Biscoe *et al.* (1984) and F. Stewart (1984).] (c) Prolactin binding to mammary gland membrane receptor protein, showing the effect of receptor protein concentration on specific prolactin binding to membranes from phase 3 (■, days 275–300), phase 2 (●, days 6–33) and phase 1 (▲) prepared and incubated with 105 000 c.p.m. of [^{125}I]oPRL in the presence or absence of 1 μg/ml unlabelled oPRL. (Redrawn from Nicholas, 1988a.)

synthesis of α-lactalbumin by mammary tissue *in vitro* is not inhibited by progesterone (Nicholas and Tyndale-Biscoe, 1985). These last authors also showed that the only hormone required for the synthesis of α-lactalbumin is prolactin at concentrations equal to the basal levels in the pregnant female (Nicholas and Tyndale-Biscoe, 1985). Prolactin injected intraductally into individual galactophores at day 15 RPY prematurely stimulated local secretion (Findlay and Renfree, 1984).

7.10.3 MAINTENANCE OF LACTATION

Prolactin in the tammar is relatively low during pregnancy except for a transient peak of 100–150 ng/ml around the time of birth and in early lactation remains at around 20–40 ng/ml at this time (Hinds and Tyndale-Biscoe, 1982b, 1985), which is the same as in non-lactating females until about 140 days of lactation. Likewise, plasma concentrations of prolactin remain relatively low in early (phase 2) lactation in all the other species in which prolactin has been measured (Figure 7.44).

However, during the second half of lactation (phase 3) the concentration rises markedly, and in the tammar remains at more than 100 ng/ml until about 280 days of lactation. The sucking stimulus is necessary to maintain this high concentration in late lactation but does not appear to be involved in early lactation (Hinds and Tyndale-Biscoe, 1985; Gordon *et al.*, 1988). Prolactin receptors increase in concentration to reach a peak at 30–34 weeks as evidenced by a significant progressive increase in the binding of prolactin ([^{125}I]-oPRL) to mammary gland membranes from quiescent, phase 1, phase 2 and phase 3 mammary glands (Figure 7.43; F.

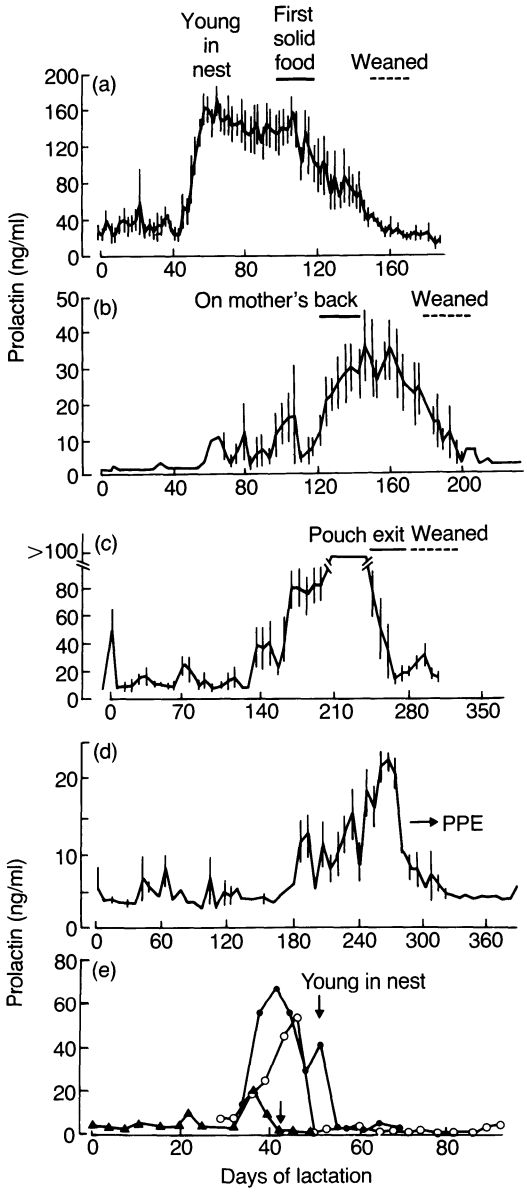


Figure 7.44 Plasma prolactin concentrations (mean ± SEM) throughout lactation in five diverse marsupials: (a) *Dasyurus viverrinus*; (b) *Trichosurus vulpecula*; (c) *Macropus rufogriseus* and (d) *Macropus eugenii*. PPE, permanent pouch exit. Data from Hinds and Tyndale-Biscoe (1985); Hinds and Merchant (1986); Hinds and Janssens (1986) and Curlewis *et al.* (1986). (e) Plasma prolactin concentrations from three individuals of *Isoodon macrourus* during lactation. ↓, pouch young lost. (a–e redrawn from Hinds, 1988.)

Stewart, 1984; Nicholas, 1988a, b). Similar prolactin profiles to the tammar have been described in the brush-tailed possum (Hinds and Janssens, 1986), the quoll (Hinds and Merchant, 1986), Bennett's wallaby (Curlewis *et al.*, 1986) and the bandicoot *I. macrourus* (Hinds, 1988). In all these species the time at which prolactin becomes elevated coincides with the increase in mammary gland size, the onset of changes in milk composition and accelerated growth of the young.

7.11 CONTROL OF MILK SECRETION AND CONCURRENT, ASYNCHRONOUS LACTATION

7.11.1 MILK COMPOSITION

One of the unusual features of lactation in marsupials is the constantly altering composition of the milk. As in all mammals, the first milk is a colostrum-type secretion, a clear, low-fat fluid with free-floating cells (Griffiths *et al.*, 1972) and with immunoglobulin G- and A-like proteins (Hindes and Mizell, 1976; Deane *et al.*, 1990). The data available from the seven or eight species studied suggest that all marsupials have a similar pattern of milk composition. The relatively dilute milk (10% solids) produced to supply the new pouch young becomes more concentrated (25–50% milk solids) as pouch life progresses (B. Green, 1984; B. Green *et al.*, 1987; B. Green and Merchant, 1988) so that the growth rates of the young are regulated by rates of milk production and its changing composition. The main constituents of marsupial milk are similar to those of eutherian mammals, but there are marked changes throughout lactation (Figure 7.45) just as the sucking regimen of the pouch young must change between the time when it is continuously attached to the teat to that when it frequently is observed off the teat (Griffiths *et al.*, 1972; B. Green *et al.*, 1980; B. Green, 1984; B. Green and Merchant, 1988).

Total solids are higher than in cow or hu-

man milk; proteins, carbohydrates and fats increase quantitatively and change qualitatively during lactation (Messer and Green, 1979). Caseins are found in marsupial milk, as well as whey proteins, but it is the whey proteins which have been best studied and show the greatest variation (Lemon and Bailey, 1966; Lemon and Barker, 1967; Lemon and Poole, 1969; S.W. Green and Renfree, 1982; Nicholas, 1988a,b). Amino acid composition also varies through lactation, and there is a correlation between sharp increases in the amount of sulphur-containing amino acids in the milk at the time of hair follicle and nail growth (Renfree *et al.*, 1981). Immunoglobulins form an important component of later marsupial milks, and the pouch young passively acquire antibodies from the milk by uptake through the gut (Yadav, 1971; Bell *et al.*, 1974; Deane and Cooper, 1988).

Concurrent, asynchronous lactation occurs

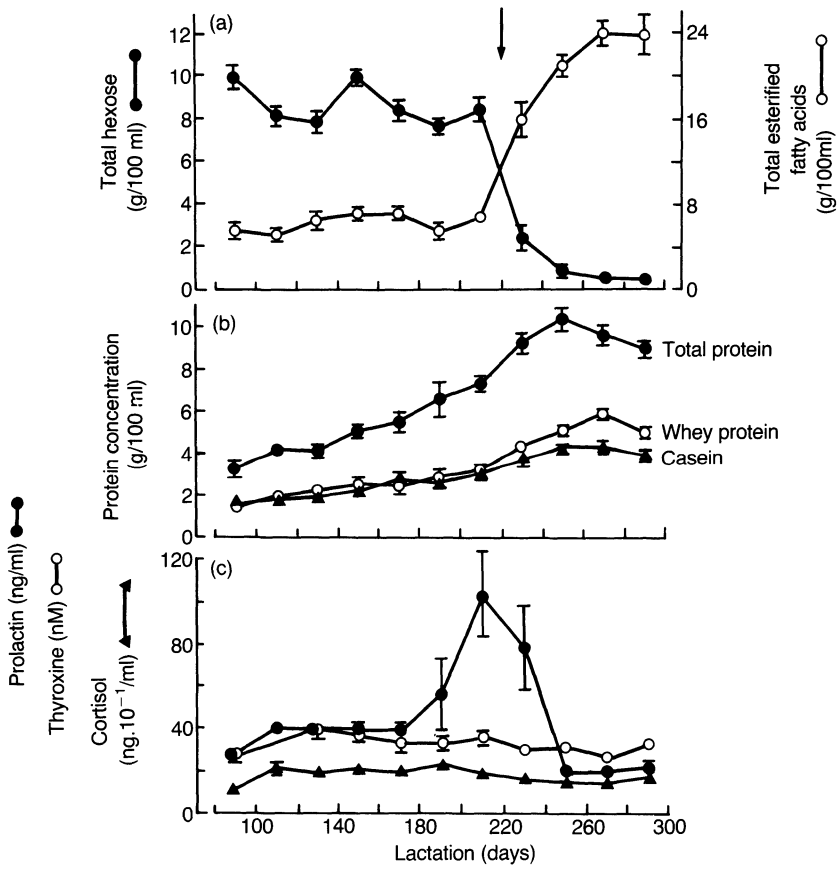


Figure 7.45 Progressive changes in milk composition and endocrine status during lactation of the tammar. (a) Total esterified fatty acid (triglyceride) and carbohydrate (total hexose) from 100 to 300 days post partum. The timing of the transition from phase 2 to phase 3 of lactation is indicated by the arrow. (b) Casein, whey, and total protein concentrations. (c) The concentration of prolactin (Hinds and Tyndale-Biscoe, 1982b), cortisol (Janssens amd Hinds, 1981) and thyroxine (Amersham radioimmunoassay kit) was measured in plasma from the same animals used for the milk analyses. (Redrawn from Nicholas, 1988a,b.)

in continuously breeding macropodids, such as the red kangaroo *M. rufus* or the agile wallaby, *M. agilis*, or even the tammar under experimental conditions. The female gives birth immediately after the pouch has been vacated by the older young, but which continues to suckle. Thus, two of the four mammary glands simultaneously secrete milk of entirely different composition. Of the two remaining glands, one may be still regressing from the previous, weaned young and the fourth regressing from the pre-partum condition. The mechanisms which control changing milk composition in marsupials and which allow the four mammary glands to differentiate and regress independently are unclear, but local factors such as sucking and milk removal are obviously important, as would be differing hormone receptor concentrations.

As mentioned earlier in this section, the induction of specific protein synthesis in phase 1 and 2 is prolactin dependent (Nicholas, 1988a,b). The response is not changed by the addition of any combination of insulin, cortisol, oestradiol-17β or thyroid hormone. In phase 3 of lactation, specific whey proteins appear in the milk, but only one, late lactation protein (LLP), has been purified (Nicholas *et al.*, 1987), and so provides a useful marker. It appears that LLP is not induced solely by the rise in prolactin soon after day 200 (Figure 7.45) because phase 2 mammary explants do not begin producing LLP when exposed to elevated prolactin (Nicholas, 1988a,b). Clearly, the mammary glands have different controls during each phase of lactation, a point reinforced by the different milk ejection responses (section 7.11.3).

Although there is as yet little information on crude growth efficiency in marsupials, their energy and growth conversions are intermediate compared to eutherian mammals (B. Green, 1984; B. Green and Merchant, 1988; B. Green *et al.*, 1988; Cork and Dove, 1989; Dove and Cork, 1989; Rose, 1987), so that the relatively long lactation and comparatively slow growth of the young is not due to inefficient conversion of milk, and must therefore be regulated/limited by the mother (B. Green and Merchant, 1988). This idea is supported by several observations. Transfer of young to pouches of larger species or mothers with larger young results in accelerated growth rates and vice versa (Merchant and Sharman, 1966; Findlay, 1982b; Findlay and Renfree, 1984; B. Green and Merchant, 1988). Only transitory effects on milk composition were produced by such transfers, but these effects were not maintained against the overriding inherent control of the cellular biochemical processes (Findlay, 1982b). It appears that changes in the composition of the milk are an intrinsic characteristic of the mammary epithelial cells and occur as the cells age (Findlay and Renfree, 1984). Such a system allows parallel but asynchronous lactations to exist independently in two adjacent glands, which can then supply different milks to physiologically different young.

7.11.2 MILK REMOVAL

The idea that contraction of the striated ilio-marsupialis muscle forcibly ejects milk from the mammary glands into the pouch young, which are considered unable to suck in the normal manner (Seiler, 1828; Morgan, 1829; Owen, 1834), continues to be expressed in the literature (Barnes, 1977) despite the fact that Enders (1966) demonstrated that milk was not expressed in the opossum following electrical stimulation of the motor nerves and associated muscles supplying the muscles of the pouch. In any event, striated muscle is apparently unnecessary for milk ejection; the glands of *Caenolestes obscurus*, the South American rat opossum, have no striated muscle at all (Griffiths and Slater, 1988).

Musculus ilio-marsupialis is a flat triangular sheet of muscle originating from the pelvis and attaching to the mammary glands; its homologue in humans is the cremaster

muscle (Tyndale-Biscoe and Renfree, 1987; Griffiths and Slater, 1988). This attachment is variable amongst species – in many, the external muscle sends branches into the secretory parenchyma of the gland. These branches diffusely invade the gland in the pouched species examined. However, in some pouchless species, such as the brown antechinus (*Antechinus stuartii*) and the dusky antechinus (*A. swainsonii*), discrete branches of the muscle attach at the base of the teats, and these may function to elevate the unprotected young from the ground when the mother is moving around (Griffiths and Slater, 1988), much as m. cremaster retracts the testis into the scrotum (Tyndale-Biscoe and Renfree, 1987). The degree to which m. iliomarsupialis penetrates the gland varies between pouched and pouchless species; in *T. vulpecula* (Bolliger and Gross, 1960; Barbour, 1963) and in the red kangaroo, *M. rufus* (Griffiths *et al.*, 1972), branches of the muscle diffusely invade the gland, and in *Tarsipes rostratus* reach each of the four teats (Griffiths and Slater, 1988). In all the pouchless species examined by Griffiths discrete branches of the muscle attach at the base of the teats. These species are *Antechinus stuartii* and *A. swainsoni*, *Dasyuroides byrnei* and *Dasysurus viverrinus* as well as the South American species *Caenolestes obscurus*. Barnes (1977) described the same arrangement in *Marmosa robinsoni*. When *A. stuartii* and *D. viverrinus* were anaesthetized the attached young hung from flaccid teats, but as the mothers regained consciousness the teats retracted and the young were again held close to the body (Griffiths and Slater, 1988).

The conclusion from these studies is that m. iliomarsupialis does not have the special function in marsupials of expressing milk and that the removal of milk in these mammals is under the same control as in eutherians, although Griffiths and Slater (1988) suggest that it is possible that striated muscle and oxytocin act synergistically in promoting contraction of the myoepithelium.

McCrady (1938), Hartman (1952) and Enders (1966) observed that neonatal opossums *D. virginiana* and *Marmosa* can suck actively, and Jurgelski (1971) using opossums and subsequently Shaw *et al.* (1988), using tammars have capitalized on this ability of neonates to suck to administer fluids containing drugs from non-distortable plastic catheter tubes. Likewise, Griffiths and Slater (1988) demonstrated that tammars, a rock wallaby, *Petrogale xanthopus*, and red kangaroo young could imbibe water or milk from rigid, non-distortable tubes. This differs from the main method of imbibition of milk in eutherians by expressing it from nipple or teat as described by Cowie (1972).

7.11.3 MILK EJECTION RESPONSE AND CONCURRENT, ASYNCHRONOUS LACTATION

It is generally agreed that milk removal is facilitated by the milk ejection reflex (Cross, 1977). The contraction of the myoepithelium investing the alveoli is effected by the humoral influence of oxytocin released from the neurohypophysis during suckling. This contraction raises intra-alveolar pressure, leading to expulsion of the milk into the ducts, and this milk can then be removed from the glands by the sucking of the young. Although, ultrastructurally, the myoepithelial network investing the mammary alveoli is similar in monotremes, marsupials and eutherians (Griffiths *et al.*, 1973), in marsupials, as noted in earlier sections, there are differing responses of the myoepithelium to exogenous hormones at the three phases of lactation as well as the structural and compositional changes. In all mammals the myoepithelial cells of the mammary gland are sensitive to oxytocin, and exogenous oxytocin causes milk ejection in marsupials as it does in eutherians. As noted above, kangaroos and wallabies are able to produce milk of two different kinds from adjacent mammary glands (Griffiths *et al.*, 1972; B. Green, 1984),

and studies of the milk ejection response also give some clues as to the manner in which this is achieved. The neurohypophysis of three American marsupials contains oxytocin, lysine vasopressin and phenypressin (Chauvet *et al.*, 1984, 1985), but in the six Australian species studied the oxytocin differs by a single amino acid – leucine is replaced by isoleucine at position 8, thus making it mesotocin (Ile^8-oxytocin) (Chauvet *et al.*, 1981). This amino acid alteration seems to have no functional significance, for heterologous (eutherian) oxytocin injected or homologous (marsupial) oxytocin released after stimulation of the presumptive oxytocinergic neurones both stimulate a milk ejection response (Lincoln and Renfree, 1981a,b).

In macropodids with concurrent asynchronous lactation, which support two young of differing ages concurrently (Figure 7.46), the question of how the milk ejection response controls the release of very different volumes of milk in the two sized glands has been addressed in the agile wallaby *M. agilis*.

Lincoln and Renfree (1981a,b) measured the intraductal pressure within the mammary gland that occurred in response to injections of oxytocin or electrical stimulation of the presumptive oxytocin neurones in the brain (Figure 7.47) and found that the basic dose – response curve is very similar to that of eutherian mammals but, early in lactation, the threshold doses of oxytocin are lower and the peak intraductal pressures obtained much higher than in late lactation (Lincoln and Renfree, 1981b).

This was clearly seen when recordings were made simultaneously in females suckling two young of different ages (Figure 7.47). In addition, there were differences in the response to electrical stimulation of the presumptive oxytocinergic neurones in the basal hypothalamus (Lincoln and Renfree, 1981a), and milk ejection also occurred when the neurones were stimulated at lower frequencies. The decline in responsiveness of the gland to oxytocin during lactation, requiring more stimulation, would thus allow the milk ejection caused by small releases of the hormone to be confined to the gland supporting the small young, while milk ejection would occur in both glands in response to the larger hormonal releases presumably triggered by the more vigorous sucking of the young-at-foot (Lincoln and Renfree, 1981a,b). This declining responsiveness as lactation

Figure 7.46 Representative stages of the pouch young and mammary gland of the agile wallaby *Macropus agilis*, during concurrent asynchronous lactation. (a) A 60-day pouch young. The enlarged nipple and gland adjacent to the attached young (arrow) is supporting a 260-day young-at-foot; (b) 210-day-old young, just about the time it makes the first excursions from the pouch. (From Findlay and Renfree, 1984, with permission.)

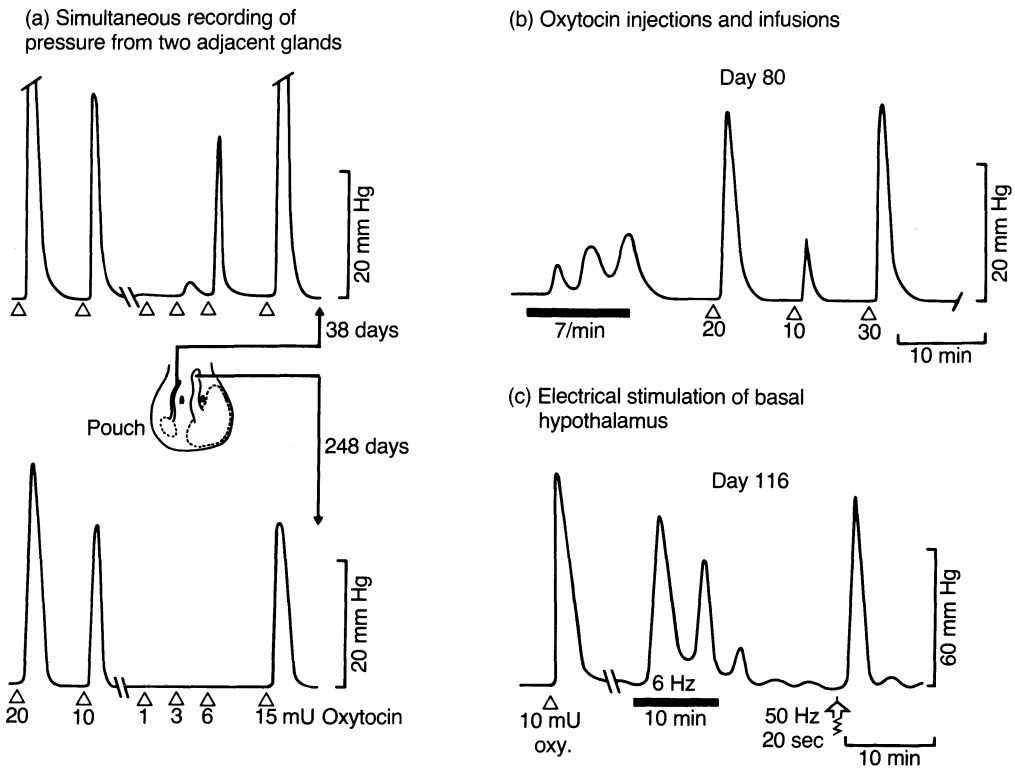


Figure 7.47 Intraductal pressure recordings taken from galactophores at different stages of lactation in agile wallabies. (a) Simultaneous recordings of intramammary pressure at 38 days and 248 days of lactation after injection (Δ) of the doses of oxytocin (mU) as shown. The 'younger' gland generated higher peak pressures and responded to lower doses of oxytocin. (b) Recording from a single galactophore at day 80 of lactation showing the response to intravenous injections (Δ) and infusions (bar) of oxytocin in the doses indicated. (c) Intramammary pressure recording at day 116 of lactation after prolonged low-frequency stimulation at 6 Hz (solid bar) compared with a short burst of high-frequency stimulation (50 Hz×20 sec. ↑) and injection of 10 mU of oxytocin (Δ). (Redrawn from Lincoln and Renfree, 1981a,b.)

progresses could be related to less effective connections between the cells of the myoepithelial network in 'older' (and larger) glands, or possibly to differences in oxytocin receptor concentrations in the cells, as has been shown for mammary gland prolactin receptors (Sernia and Tyndale-Biscoe, 1979; Tyndale-Biscoe *et al.*, 1984; Figure 7.43). Thus, milk ejection can occur semi-independently in adjacent glands during concurrent, asynchronous lactation.

7.11.4 CONTROL OF MILK SECRETION

Local intrinsic control and developmentally regulated mechanisms of the mammary epithelium appear to be responsible for the complex biochemical and physiological changes which occur in marsupial mammary glands through lactation (Findlay and Renfree, 1984; Nicholas, 1988a). Intraductal injection of prolactin causes a local secretory response in phase 1 of lactation (Findlay, 1982b; Findlay and Renfree, 1984), but specific proteins such as LLP are only induced by prolactin in phase 3 and not phase 2 of lactation (Nicholas,

1988a,b). Similarly, during phase 2 of lactation the sucking stimulus does not apparently cause a prolactin release, because neither removal of pouch young nor injection of bromocriptine depresses the low (< 40 ng/ml) concentrations of plasma prolactin (Hinds and Tyndale-Biscoe, 1982b, 1985; Tyndale-Biscoe and Hinds, 1984; Curlewis *et al.*, 1986; Gordon *et al.*, 1988), nor does denervation of the teat stop milk production (Renfree, 1979). Developmental age of the mammary gland overrides the effects of an experimentally altered sucking interval (rate) and volume of milk withdrawal so that the milk composition shows only transitory changes (Findlay, 1982b, Findlay and Renfree, 1984). These changes may be mediated by alterations in hormone receptor concentrations, such as are suggested to explain the decline in responsiveness of the mammary myoepithelium to mesotocin as lactation proceeds (Lincoln and Renfree, 1981a,b), as has been shown for mammary prolactin receptors (Tyndale-Biscoe *et al.*, 1984). This declining responsiveness in the milk ejection response could also be related to less effective connections between the cells of the myoepithelial network and older and larger glands (Lincoln and Paisley, 1982; Findlay and Renfree, 1984).

Support for the idea that the changes which occur during lactation in marsupials are internally controlled within the individual mammary glands comes from the interesting recent studies by Peaker, Wilde and colleagues on lactating goats. They have found evidence that milk secretion is modulated by local chemical feedback inhibition (autocrine control) by an inhibitor (a protein of 10–30 kDa) present in the whey protein fraction (Henderson and Peaker, 1984; Peaker and Wilde, 1987; Wilde *et al.*, 1987, 1988; G.M. Stewart *et al.*, 1988). In other words, the secretory cells of the mammary gland produce an inhibitor (or proinhibitor) of their own activity, such that more frequent removal of the protein during an increase in milking frequency enhances milk secretion. The gland does not become refractory to these autocrine stimuli, and the enhanced secretory rate is maintained for as long as the stimulus of frequent milking is applied (Peaker and Wilde, 1987). In ruminants, local production of prostaglandins can also inhibit the induction of milk synthesis (Maule Walker, 1984). Mechanisms like these may well operate in marsupial mammary glands, but although the data on autocrine control of milk secretion provide an explanation for the production of differing volumes of milk in adjacent glands, which is clearly controlled by the sucking activity of the young, the question of the control of milk compositional changes in adjacent mammary glands remains more open. These latter changes appear to be controlled by the secretory 'age' of the mammary gland and may therefore be regulated by the mother.

7.12 ADAPTIVE SIGNIFICANCE OF THE MARSUPIAL MODE OF PREGNANCY AND LACTATION

Marsupials have a different mode of reproduction from eutherian mammals, in that they give birth to small altricial young after a relatively short gestation. However, with the growing body of knowledge on marsupial reproduction it is clear that marsupials share with eutherians the same physiological complexities that control reproduction (Tyndale-Biscoe and Renfree, 1987). Progesterone is the essential hormone of pregnancy, oestradiol of ovulation and oestrus, and parturition is associated with a rise in prostaglandins and prolactin. Seasonal factors, notably photoperiod, influence the annual cycle of reproduction mediated by the pineal hormone melatonin (Tyndale-Biscoe *et al.*, 1986). Their lactational physiology is the most sophisticated of any mammal, and it is here that the greatest differences are apparent between the marsupial and eutherian mode of reproduction (Renfree, 1983). The teat is the umbi-

lical cord of the marsupial, and they have elaborated lactation instead of extending the period of intrauterine development with all its attendant hazards.

There has been a great deal of speculation as to which method is evolutionally superior; in general, perhaps because of a lack of knowledge, the marsupial mode has been assumed to be the 'primitive' and, by inference, inferior pattern. However, when the reproductive processes of the three groups of mammals – monotremes, marsupials and eutherians – are compared, many features are synapomorphic (derived) characters held in common by all three (Tyndale-Biscoe and Renfree, 1987). These are Graafian follicles, functional corpora lutea, bilaminar blastocysts, uterine secretion, yolk sac placentae, mammary glands and lactation. While monotremes retain some plesiomorphic (primitive) characters, such as egg laying, which show an early divergence from the stock leading to marsupials and eutherians, comparisons between marsupials and eutherians suggest a dichotomy in development from a common ancestral group of therian mammals, not a derivation of one from the other (Tyndale-Biscoe and Renfree, 1987). Most of the apomorphic characters of marsupial reproduction are associated with the greater emphasis on lactation than gestation.

Few attempts have been made to measure the cost of lactation (Rose, 1987; B. Green and Merchant, 1988; B. Green *et al.*, 1988). One way is to compare the maternal investment and the energy required to rear a young to weaning, and this has recently been done (Cork and Dove, 1989; Dove and Cork, 1989). Using the tammar and the sheep as models, it appears that the pattern of milk intake is similar in both types of mammals if eutherian lactation is compared only with the post-pouch exit life of marsupials. During pouch life, the marsupial pattern of milk energy intake is similar to that of the energy deposition in the eutherian fetus during pregnancy (Cork and Dove, 1989; Dove and Cork, 1989). These authors conclude that the energy cost of lactation in the tammar can thus be regarded as equivalent to the sum of the energy costs of pregnancy and lactation in the sheep. Furthermore, there are not large differences between these two herbivores in terms of weight-related allocation of energy to reproduction (Cork and Dove, 1989). Thus the marsupial strategy of spreading the offspring's intake of energy over a long lactation appears to be an important adaptation for a **small** grazing mammal living in a relatively aseasonal environment which is not faced with brief periods of seasonal food abundance. Thus, it can afford to have an extensive as opposed to an intensive grazing time, so giving it a relative ecological advantage. These important analyses of Cork and Dove show that the energy requirements for reproduction and development in their model marsupial, the tammar, fall within the range of comparable eutherians. Furthermore, they confirm that the transfer of energy in the form of milk is limited by the mother (Dove and Cork, 1989), just as the mother limits the period of pouch occupation in the Tasmanian rat kangaroos by dramatically constricting her pouch (Rose, 1986, 1987). These data are not consistent with the suggestion of Lillegraven *et al.* (1987) that energy transfer between mother and young during reproduction is less efficient in marsupials than in eutherian mammals, but show that these are highly derived patterns, and support the contention of Tyndale-Biscoe and Renfree (1987) that the major differences seen in the mode of reproduction in living mammals today evolved in response to the metabolic requirements of increasing body size and the ecological constraints imposed during the great adaptive radiations of mammals during the Tertiary epoch into new niches and ways of life.

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REFERENCES

Alcorn, G.T. and Robinson, E.S. (1983) Germ cell development in female pouch young of the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **67**, 319–325.

Amoroso, E.C., Heap, R.B. and Renfree, M.B. (1980) Hormones and the evolution of viviparity, in *Hormones and Evolution*, Vol. 2 (ed. E.J.W. Barrington), Academic Press, London, pp. 925–989.

Andrewartha, H.G. and Barker, S. (1969) Introduction to a study of the ecology of the Kangaroo Island wallaby *Protemnodon eugenii* (Desmarest) within Flinders Chase, Kangaroo Island, South Australia. *Transactions – Royal Society of South Australia*, **93**, 127–132.

Archer, M. (1984) Origins and early radiations of mammals, in *Vertebrate Zoogeography and Evolution in Australasia*, (eds. M. Archer and G. Clayton), Perth, Western Australia. Hesperin Press, pp. 477–516.

Bancroft, B.J. (1973) Embryology of *Schoinobates volans* (Kerr) (Marsupialia:Petauridae). *Australian Journal of Zoology*, **21**, 33–52.

Barbour, R.A. (1963) The musculature and limb plexuses of *Trichosurus vulpecula*. *Australian Journal of Zoology*, **11**, 488–610.

Barnes, R.D. (1977) The special anatomy of *Marmosa robinsoni*, in *The Biology of Marsupials*, (ed. D. Hunsaker II), Academic Press, New York, pp. 387–413.

Baudinette, R.V., Runciman, S.I.C., Frappell, P.F. and Gannon, B.J. (1988) Development of the marsupial cardiorespiratory system, in *The Developing Marsupial: Models for Biomedical Research*, (eds C.H. Tyndale-Biscoe and P.A. Janssens), Springer Verlag, Berlin, pp. 132–147.

Bell, R.G., Stephens C.J. and Turner, K.J. (1974) Marsupial immunoglobins: An immunoglobulin molecule resembling eutherian IgA in serum and secretions of *Setonix brachyurus* (quokka). *Journal of Immunology*, **113**, 371–378.

Berger, P.J. (1966) Eleven-month 'Embryonic Diapause' in a marsupial. *Nature*, **211**, 435–436.

Berger, P.J. (1970) Reproductive biology of the tammar wallaby, *Macropus eugenii*. PhD Thesis, Tulane University. *Dissertation Abstracts International*, **31B**, 3760–3761.

Berger, P.J. and Sharman, G.B. (1969a) Embryonic diapause initiated without the suckling stimulus in the wallaby, *Macropus eugenii*. *Journal of Mammalogy*, **50**, 630–632.

Berger, P.J. and Sharman, G.B. (1969b) Progesterone-induced development of dormant blastocysts in the tammar wallaby, *Macropus eugenii* (Desmarest: Marsupialia). *Journal of Reproduction and Fertility*, **20**, 201–210.

Bolliger, A. and Gross, R. (1960) Nutrition of the marsupial suckling. *Australian Journal of Science*, **22**, 292–293.

Bowley, E.A. (1939) Delayed fertilisation in *Dromicia*. *Journal of Mammalogy*, **20**, 499.

Bradshaw, F.J. and Bradshaw, S.D. (1992) Estradiol-17β secretion by the newly formed corpus luteum of a macropodid marsupial, the quokka (*Setonix brachyurus*). *General and Comparative Endocrinology*, **87**, 425–435.

Bradshaw, S.D., McDonald, I.R., Hahnel, R. and Heller, H. (1975) Synthesis of progesterone by the placenta of a marsupial. *Journal of Endocrinology*, **65**, 451–452.

Breed, W.G. and Leigh, C.M. (1988) Morphological observations on sperm–egg interactions during *in vivo* fertilization in the dasyurid marsupial, *Sminthopsis crassicaudata*. *Gamete Research*, **19**, 131–149.

Breed, W.G. and Leigh, C.M. (1990) Morphological changes in the oocyte and its surrounding vestments during *in vivo* fertilization in the dasyurid marsupial, *Sminthopsis crassicaudata*. *Journal of Morphology*, **204**, 177–196.

Breed, W.G., Leigh, C.M. and Bennett, J.H. (1989)

Sperm morphology and storage in the female reproductive tract of the fat-tailed dunnart, *Sminthopsis crassicaudata* (Marsupialia:Dasyuridae). *Gamete Research*, **23**, 61–75.

Bryant, S.L. and Rose, R.W. (1986) The growth and role of the corpus luteum throughout delayed gestation in the marsupial, *Potorous tridactylus*. *Journal of Reproduction and Fertility*, **76**, 409–414.

Burns, R.K. (1961) Role of hormones in differentiation, in *Sex and Internal Secretions*, Vol. 1, Chapter 2 (ed. W.C. Young), Williams & Wilkins, Baltimore, pp. 76–158.

Cake, M.H., Owen, F.J. and Bradshaw, S.D. (1980) Difference in concentration of progesterone in the plasma between pregnant and non-pregnant quokkas (*Setonix brachyurus*). *Journal of Endocrinology*, **84**, 153–158.

Calaby, J.H. and Poole, W.E. (1971) Keeping kangaroos in captivity. *International Zoo Yearbook*, **11**, 5–12.

Call, R.N., Catling, P.C. and Janssens, P.A. (1980) Development of the adrenal gland in the tammar wallaby, *Macropus eugenii* (Desmarest) (Marsupialia:Macropodidae). *Australian Journal Zoology*, **28**, 249–259.

Callard, G.V., Petro, Z. and Tyndale-Biscoe, C.H. (1982) Aromatase activity in marsupial brain, ovaries and adrenals. *General and Comparative Endocrinology*, **46**, 541–546.

Cannon, J.R., Bakker, H.R., Bradshaw, S.D. and McDonald, I.R. (1976) Gravity as the sole navigational aid to the newborn quokka. *Nature (London)*, **259**, 42.

Catling, P.C. and Sutherland, R.L. (1980) Effect of gonadectomy, season, and the presence of females on plasma testosterone, luteinizing hormone, and follicle stimulating hormone levels in male tammar wallabies (*Macropus eugenii*). *Journal of Endocrinology*, **86**, 25–33.

Catling, P.C. and Vinson, G.P. (1976) Adrenocortical hormones in the neonate and pouch young of the tammar wallaby, *Macropus eugenii*. *Journal of Endocrinology*, **69**, 447–448.

Chauvet, M.T., Hurpet, D., Chauvet, J. and Acher, R. (1981) A reptilian neurohypophysial hormone, mesotocin (Ile^8–oxytocin), in Australian marsupials. *FEBS Letters*, **129**, 120–122.

Chauvet, J., Hurpet, D., Chauvet, M.T., and Acher, R. (1984) Divergent neuropeptide evolutionary drifts between American and Australian marsupials. *Bioscience Reports*, **4**, 245–252.

Chauvet, J., Hurpet, D., Colne, T., Michel, G., Chauvet, M.T. and Acher, R. (1985) Neurohypophyseal hormones as evolutionary tracers: identification of oxytocin, lysine vasopressin, and arginine vasopressin in two South American opossums (*Didelphis marsupialis* and *Philander opossum*). *General and Comparative Endocrinology*, **57**, 320–328.

Clark, M.J. (1967). Pregnancy in the lactating pigmy possum, *Cercartetus concinnus*. *Australian Journal of Zoology*, **15**, 673–683.

Clark, M.J. (1968) Termination of embryonic diapause in the red kangaroo, *Megaleia rufa*, by injection of progesterone or oestrogen. *Journal of Reproduction and Fertility*, **15**, 347–355.

Cook, B. and Nalbandov, A.V. (1968) The effect of some pituitary hormones on progesterone synthesis *in vitro* by the luteinized ovary of the common opossum (*Didelphis marsupialis virginiana*). *Journal of Reproduction and Fertility*, **15**, 267–275.

Cook, B., Sutterlin, N.S., Graber, J.W. and Nalbandov, A.V. (1977) Synthesis and action of gonadal steroids in the American opossum *Didelphis marsupialis*, in *Reproduction and Evolution*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), Australian Academy of Science, Canberra, pp. 253–254.

Cork, S.J. and Dove, H. (1989) Lactation in the tammar wallaby (*Macropus eugenii*). II. Intake of milk components and maternal allocation of energy. *Journal of Zoology*, **219**, 385–397.

Cowie, A.T. (1972) Lactation and its hormonal control, in *Reproduction in Mammals*, Vol. 3. (eds. C.R. Austin and R.V. Short), Cambridge University Press, pp. 106–143.

Cowie, A.T., Forsyth, I.A. and Hart, I.C. (1980) Hormonal Control of Lactation. *Monographs in Endocrinology*, Springer-Verlag, Berlin.

Cross, B.A. (1977) Comparative physiology of milk removal. *Symposium of the Zoological Society (London)*, **41**, 193–210.

Curlewis, J.D. and Loudon, A.S.I. (1988) Effects of photoperiod on the 24-hour melatonin profiles of the Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Journal of Pineal Research* **5**, 373–383.

Curlewis, J.D. and Loudon, A.S.I. (1989) The role of refractoriness to long day length in the annual reproductive cycle of the female Bennett's wallaby *Macropus rufogriseus rufogriseus*). *Journal of Experimental Zoology*, **252**, 200–206.

Curlewis, J.D. and Stone, G.M. (1986) Effects of oestradiol, the oestrous cycle and pregnancy on

weight, metabolism and cytosol receptors in the uterus of the brush-tail possum (*Trichosurus vulpecula*). *Journal of Endocrinology*, **108**, 201–210.

Curlewis, J.D., Axelson, M. and Stone, G.M. (1985) Identification of the major steroids in ovarian and adrenal venous plasma of the brush-tail possum (*Trichosurus vulpecula*) and changes in the peripheral plasma level of oestradiol and progesterone during the reproductive cycle. *Australian Journal of Biological Science*, **38**, 313–326.

Curlewis, J.D., White, A.S., Loudon, A.S.I. and McNeilly, A.S. (1986) Effects of lactation and season on plasma prolactin concentrations and response to bromocriptine during lactation in the Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Journal of Endocrinology*, **110**, 59–66.

Curlewis, J.D., Renfree, M.B., Sheldrick, E.L. and Flint, A.P.F. (1988) Mesotocin and luteal function in macropodid marsupials. *Journal of Endocrinology*, **117**, 367–372.

Daniel, J.C. (1970) Coincidence of embryonic growth and uterine protein in the ferret. *Journal of Embryology and Experimental Morphology*, **24**, 305–312.

Daniel, J.C. (1980) Factors influencing uteroglobin synthesis, in *Steroid Induced Uterine Proteins*, (ed. M. Beato), Elsevier/North Holland Biomedical Press, Amsterdam, pp. 87–103.

Daniel, J.C., Jetton A.E., and Chilton, B.S. (1984) Prolactin as a factor in the uterine response to progesterone in rabbits. *Journal of Reproduction and Fertility*, **72**, 443–452.

Deane, E.M. and Cooper, D.W. (1988) Immunological development of pouch young marsupials, in *The Developing Marsupial: Models for Biomedical Research*, (eds C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin, pp. 190–199.

Deane, E.M., Cooper, D.W. and Renfree, M.B. (1990) Immunoglobulin G levels in fetal and newborn tammar wallabies (*Macropus eugenii*). *Journal of Reproduction, Fertility and Development*, **2**, 369–375.

Denker, H.W. and Tyndale-Biscoe, C.H. (1986) Embryo implantation and proteinase activities in a marsupial (*Macropus eugenii*). *Cell and Tissue Research*, **246**, 279–91.

Dove, H. and Cork, S.J. (1989) Lactation in the tammar wallaby (*Macropus eugenii*). I. Milk consumption and the algebraic description of the lactation curve. *Journal of Zoology (London)*, **219**, 385-339.

Enders, A.C. (1966) The reproductive cycle of the nine-banded armadillo (*Dasyurus novemcinctus*). *Symposium of the Zoological Society (London)*, **15**, 295–310.

Enders, A.C. (1973) Cytology of the corpus luteum. *Biology of Reproduction*, **8**, 158–182.

Evans, S.M., Tyndale-Biscoe, C.H. and Sutherland, R.L. (1980) Control of gonadotrophin secretion in the female tammar wallaby (*Macropus eugenii*). *Journal of Endocrinology*, **86**, 13–23.

Fadem, B.H. and Rayve, R.S. (1985) Characteristics of the oestrous cycle and influence of social factors in grey short-tailed opossums (*Monodelphis domestica*). *Journal of Reproduction and Fertility*, **73**, 337–342.

Fadem, B.H. and Tesoriero, J.V. (1986) Inhibition of testicular development and feminization of the male genitalia by neonatal estrogen treatment in a marsupial. *Biology of Reproduction*, **34**, 771–776.

Findlay, L. (1982a) The mammary glands of the tammar wallaby (*Macropus eugenii*) during pregnancy and lactation. *Journal of Reproduction and Fertility*, **65**, 59–66.

Findlay, L. (1982b) Lactation in the marsupial, *Macropus eugenii. PhD Thesis*, Murdoch University, Perth.

Findlay, L. and Renfree, M.B. (1984) Growth, development and secretion of the mammary gland of macropodid marsupials. *Symposium of the Zoological Society (London)*, **51**, 403–432.

Findlay, L., Ward, K.L. and Renfree, M.B. (1983) Mammary gland lactose, plasma progesterone and lactogenesis in the marsupial, *Macropus eugenii*. *Journal of Endocrinology*, **97**, 425–436.

Fleet, I.R., Goode, J.A., Hamon, M.H., Laurie, M.S., Linzell, J.L. and Peaker, M.H. (1975) Secretory activity of goat mammary glands during pregnancy and the onset of lactation. *Journal of Physiology (London)*, **251**, 763–773.

Fleming, M.W. and Harder, J.D. (1981a) Uterine histology and reproductive cycles in pregnant and non-pregnant opossums, *Didelphis virginiana*. *Journal of Reproduction and Fertility*, **63**, 21–24.

Fleming, M.W. and Harder, J.D. (1981b) Effect of pregnancy on uterine constituents of the Virginia opossum. *Comparative Biochemistry and Physiology*, **69A**, 337–339.

Fletcher, T.P. (1983) Endocrinology of reproduction in the dasyurid marsupial, *Dasyuroides*

byrnei (*Spencer*). *PhD Thesis*, La Trobe University, Melbourne.

Fletcher, T.P. (1989a) Luteinizing hormone in the kowari, *Dasyuroides byrneii* (Marsupialia:Dasyuridae), during the oestrous cycle and pregnancy, and the effects of gonadectomy in male and female. *Reproduction Fertility and Development*, **1**, 55–64.

Fletcher, T.P. (1989b) Plasma progesterone and body weight in the pregnant and non-pregnant kowari, *Dasyuroides byrnei* (Marsupialia:Dasyuridae). *Reproduction Fertility and Development*, **1**, 65–74.

Fletcher, T.P. and Renfree, M.B. (1988) Effects of corpus luteum removal on progesterone, oestradiol-17β and LH in early pregnancy of the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **83**, 185–191.

Fletcher, T.P., Jetton, A.E. and Renfree, M.B. (1988) Influence of progesterone and oestradiol-17β on blastocysts of the tammar wallaby during seasonal diapause. *Journal of Reproduction and Fertility*, **83**, 193–200.

Fletcher, T.P., Shaw, G. and Renfree, M.B. (1990) Parturition in the tammar wallaby: effects of bromocriptine. *Reproduction Fertility and Development*, **2**, 79–88.

Flint, A.P.F. and Renfree, M.B. (1982) Oestradiol-17β in the circulation during seasonal reactivation of the diapausing blastocyst in a wild population of tammar wallabies, *Macropus eugenii*. *Journal of Endocrinology*, **95**, 293–300.

Flynn, T.T. (1923) The yolk sac and allantoic placenta in *Perameles*. *Quarterly Journal of Microscopical Science*, **67**, 123–182.

Flynn, T.T. (1930) The uterine cycle of pregnancy and pseudopregnancy as it is in the diprotodont marsupial *Bettongia cuniculus* with notes on other reproductive phenomena in this marsupial. *Proceedings of Linnean Society of New South Wales*, **55**, 506–531.

Gadsby, J.E., Heap, R.B. and Burton, R.D. (1980) Oestrogen production by blastocyst and early embryonic tissue of various species. *Journal of Reproduction and Fertility*, **60**, 409–417.

Gallo, A.B., Licht, P., Farmer, S.W., Papkoff, H. and Hawkins, J. (1978) Fractionation and biological actions of pituitary gonadotropins from a marsupial, the wallaby *Macropus eugenii*. *Biology of Reproduction*, **19**, 680–687.

Gemmell, R.T. (1979) The fine structure of the luteal cells in relation to the concentration of progesterone in the plasma of the lactating bandicoot, *Isoodon macrourus* (Marsupialia:Peramelidae). *Australian Journal of Zoology*, **27**, 501–510.

Gemmell, R.T. (1981) The role of the corpus luteum of lactation in the bandicoot *Isoodon macrourus* (Marsupialia:Peramelidae). *General and Comparative Endocrinology*, **44**, 13–19.

Gemmell, R.T. (1984) Plasma concentrations of progesterone and 13,14-dihydro–15–keto–prostaglandin $F_{2\alpha}$ during regression of the corpora lutea of lactation in the bandicoot (*Isoodon macrourus*). *Journal of Reproduction and Fertility*, **72**, 295–299.

Gemmell, R.T. (1986) Lung development in the marsupial bandicoot, *Isoodon macrourus*. *Journal of Anatomy*, **148**, 193–204.

Gemmell, R.T. (1987) Effect of melatonin and removal of pouch young on the seasonality of birth in the marsupial possum, *Trichosurus vulpecula*. *Journal of Reproduction and Fertility*, **80**, 301–307.

Gemmell, R.T. and Little, G.J. (1982) The structure of the lung of the newborn marsupial bandicoot, *Isoodon macrourus*. *Cell and Tissue Research*, **223**, 445–453.

Gemmell, R.T. and Nelson, J. (1988a) The ultrastructure of the pituitary and the adrenal gland of three newborn marsupials (*Dasyurus hallucatus*, *Trichosurus vulpecula* and *Isoodon macrourus*). *Anatomy and Embryology*, **177**, 395–402.

Gemmell, R.T. and Nelson, J. (1988b) The ultrastructure of the olfactory system of three newborn marsupial species. *Anatomical Record*, **221**, 655–662.

Gemmell, R.T. and Nelson, J. (1988c) The ultrastructure of the lung of two newborn marsupial species, the northern native cat, *Dasyurus hallucatus*, and the brushtail possum, *Trichosurus vulpecula*. *Cell and Tissue Research*, **252**, 683–685.

Gemmell, R.T. and Nelson, J. (1989d) The vestibular system of the newborn marsupial cat, *Dasyurus hallucatus*. *Anatomical Record*, **225**, 203–208.

Gemmell, R.T. and Rose, R.W. (1989a) The senses involved in movement of some newborn Macropodoidea and other marsupials from cloaca to pouch, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P. Jarman, I.D. Hume and G. Grigg), Surrey Beatty and Sons Pty. Ltd., Sydney. pp. 339–47.

Gemmell, R.T. and Rose, R.W. (1989b) Organ development in some newborn marsupials with particular reference to the rat kangaroos, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P.

Jarman, I.D. Hume and G. Grigg), Surrey Beatty & Sons Pty. Ltd., Sydney, pp. 349–354.

Gemmell, R.T. and Selwood, L. (1993) Structural development in the newborn marsupial, the stripe-faced dunnart, *Sminthopsis macroura*. *Acta Anatomica*, (in press).

Gemmell, R.T., Jenkin, G. and Thorburn, G.D. (1980) Plasma concentrations of progesterone and 13, 14–dihydro–15–keto–prostaglandin $F_{2\alpha}$at parturition in the bandicoot, *Isoodon macrourus*. *Journal of Reproduction and Fertility*, **60**, 253–256.

Gemmell, R.T., Singh-Asa, P., Jenkin, G. and Thorburn, G.D. (1982) Ultrastructural evidence for steroid-hormone production in the adrenal of the marsupial, *Isoodon macrourus*, at birth. *Anatomical Record*, **203**, 505–512.

Gemmell, R.T., Hughes, R.L. and Jenkin, G. (1987) Comparative studies on the hormonal profiles of progesterone and prostaglandin F metabolite in the possum, *Trichosurus vulpecula*, in *Possums and Opossums: Studies in evolution*, (ed. M. Archer), Royal Zoological Society of New South Wales, Sydney, Australia.

Gemmell, R.T., Peters, B. and Nelson, J. (1988) Ultrastructural identification of Merkel cells around the mouth of the newborn marsupial. *Anatomy and Embryology*, **177**, 403–408.

George, F.W., Hodgins, M.B. and Wilson, J.D. (1985) The synthesis and metabolism of gonadal steroids in pouch young of the opossum, *Didelphis virginiana*. *Endocrinology*, **116**, 1145–50.

Godfrey, G.K. (1975) A study of oestrus and fecundity in a laboratory colony of mouse opossums, *Marmosa robinsoni*. *Journal of Zoology (London)*, **175**, 541–555.

Gordon, K., Fletcher, T.P. and Renfree, M.B. (1988) Reactivation of the quiescent corpus luteum and diapausing embryo after temporary removal of the sucking stimulus in the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **83**, 401–406.

Green, B. (1984) Composition of milk and energetics of growth in marsupials. *Symposium of the Zoological Society (London)*, **51**, 369–387.

Green, B. and Merchant, J.C. (1988) The composition of marsupial milk, in *The Developing Marsupial. Models for Biomedical Research*, Chapter 4 (eds C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin, Heidelberg.

Green, B., Newgrain K., and Merchant (1980) Changes in milk composition during lactation in the tammar wallaby (*Macropus eugenii*). *Australian Journal Biological Science*, **33**, 35–42.

Green, B., Merchant, J.C. and Newgrain K. (1987) Milk composition in the eastern quoll, *Dasyurus viverrinus* (Marsupialia:Dasyuridae). *Australian Journal of Biological Science* **40**, 379–387.

Green, B., Merchant, J.C. and Newgrain, K. (1988) Milk consumption and energetics of growth in pouch young of the tammar wallaby (*Macropus eugenii*). *Australian Journal of Zoology*, **36**, 217–229.

Green, S.W. and Renfree, M.B. (1982) Changes in the milk proteins during lactation in the tammar wallaby (*Macropus eugenii*). *Australian Journal of Biological Science*, **35**, 145–152.

Griffiths, M. and Slater, E. (1988) The significance of striated muscle in the mammary glands of marsupials. *Journal of Anatomy*, **156**, 141–156.

Griffiths, M., McIntosh, D.L. and Leckie, R.M.C. (1972) The mammary glands of the red kangaroo, with observations on the fatty acid components of the milk triglycerides. *Journal of Zoology (London)*, **166**, 265–275.

Griffiths, M., Elliott, M.A., Leckie, R.M.C. and Schoefl, G.I. (1973) Observations of the comparative anatomy and ultrastructure of mammary glands, and on the fatty acids of the triglycerides in platypus and echidna milk fats. *Journal of Zoology (London)*, **169**, 255–279.

Hall L.S. (1988) The embryology of the marsupial, *Isoodon macrourus*, with particular reference to fetation. *PhD Thesis*, University of Queensland.

Hall, L.S. and Hughes, R.L. (1985) The embryological development and cytodifferentiation of the anterior pituitary in the marsupial, *Isoodon macrourus*. *Anatomy and Embryology*, **172**, 353–363.

Hall, L.S. and Hughes, R.L. (1987) An evolutionary perspective of structural adaptations for environmental perception and utilization by the neonatal marsupials *Trichosurus vulpecula* and *Didelphis virginiana*, in *Possums and opossums*, (ed. M. Archer), *Royal Zoology Society, New South Wales*, Sydney. pp. 257–771.

Harder, J.D. and Fleming, M.W. (1981) Estradiol and progesterone profiles indicate a lack of endocrine recognition of pregnancy in the opossum. *Science*, **212**, 1400–1402.

Harder, J.D., Hinds, L.A., Horn, C.A. and Tyndale-Biscoe, C.H. (1984) Oestradiol in follicular fluid and in utero-ovarian venous and peripheral plasma during parturition and post-

partum oestrus in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **72**, 551–558.

Harder, J.D., Hinds, L.A., Horn, C.A. and Tyndale-Biscoe, C.H. (1985) Effects of removal in late pregnancy of the corpus luteum, Graafian follicle or ovaries on plasma progesterone, oestradiol, LH, parturition and post-partum oestrus in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **75**, 449–459.

Hartman, C.G. (1923). The oestrous cycle in the opossum. *American Journal of Anatomy*, **32**, 353–421.

Hartman, C.G. (1925). The interruption of pregnancy by ovariectomy in the aplacental opossum: a study in the physiology of implantation. *American Journal of Physiology* ,**71**, 436–454.

Hartman, C.G. (1928) The breeding season of the opossum (*Didelphis virginiana*) and the rate of intrauterine and postnatal development. *Journal of Morphology and Physiology*, **46**, 143–215.

Hartman, C.G. (1952). *Possums*, University of Texas Press, Austin.

Heap, R.B., Renfree, M.B. and Burton, R.D. (1980) Steroid metabolism in the yolk sac placenta and endometrium of the tammar wallaby, *Macropus eugenii*. *Journal of Endocrinology*, **87**, 339–349.

Hearn, J.P. (1973) Pituitary inhibition of pregnancy. *Nature*, **241**, 207–208.

Hearn, J.P. (1974) The pituitary gland and implantation in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **39**, 235–241.

Hearn, J.P. (1975) Hypophysectomy of the tammar wallaby, *Macropus eugenii*: surgical approach and general effects. *Journal of Endocrinology*, **64**, 403–416.

Heller, H. (1973) The effects of oxytocin and vasopressin during the oestrous cycle and pregnancy on the uterus of a marsupial species, the quokka (*Setonix brachyurus*). *Journal of Endocrinology*, **58**, 657–671.

Henderson, A.J. and Peaker, M. (1984) Feedback control of milk secretion in the goat by a chemical in milk. *Journal of Physiology (London)*, **351**, 39–45.

Hill, J.P. (1900) Contributions to the morphology and development of the female urogenital organs in the Marsupialia. *Proceedings of the Linnean Society of New South Wales*, **25**, 519–532.

Hill, J.P. and O'Donoghue, C.H. (1913) The reproductive cycle in the marsupial *Dasyurus viverrinus*. *Quarterly Journal of Microscopical Science*, **59**, 133–174.

Hindes, R.D. and Mizell, M. (1976) The origin of immunoglobulins in opossum 'embryos'. *Developmental Biology*, **53**, 49–61.

Hinds, L.A. (1983) Progesterone and prolactin in marsupial reproduction. *PhD Thesis*, Australian National University, Canberra, Australia.

Hinds, L.A. (1988) Hormonal control of lactation, in *The Developing Marsupial. Models for Biomedical Research*, Chapter 5 (eds C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin.

Hinds, L.A. (1989a) Plasma progesterone through pregnancy and the estrous cycle in the Eastern Quoll *Dasyurus viverrinus*. *General and Comparative Endocrinology*, **75**, 110–117.

Hinds, L.A. (1989b) Pregnancy deferred: An unusual role for prolactin in a marsupial. *Serono Symposium, USA*, (ed. Koji Yoshinaga), Adams Publishing Group, Boston, Massachusetts, pp. 201–207.

Hinds, L.A. (1989c) Morning pulse of prolactin maintains seasonal quiescence in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **87**, 735–744.

Hinds, L.A. (1990) The control of pregnancy, parturition and luteolysis in marsupials. *Reproduction Fertility and Development*, **2**, 535–551.

Hinds, L.A. and den Ottolander, R.C. (1983) Effect of changing photoperiod on peripheral plasma prolactin and progesterone concentrations in the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **69**, 631–639.

Hinds, L.A. and Janssens, P.A. (1986) Changes in prolactin in peripheral plasma during lactation in the brushtail possum, *Trichosurus vulpecula*. *Australian Journal of Biological Science*, **39**, 171–178.

Hinds, L.A. and Merchant, J.C. (1986) Plasma prolactin concentrations throughout lactation in the eastern quoll, *Dasyurus viverrinus* (Marsupialia:-Dasyuridae). *Australian Journal of Biological Science*, **39**, 179–186.

Hinds, L.A. and Selwood, L. (1990) Plasma progesterone concentrations during pregnancy in the dasyurid marsupial, *Antechinus stuartii*: relationship with differentiation of the embryo. *Reproduction Fertility and Development*, **2**, 61–70.

Hinds, L.A. and Tyndale–Biscoe, C.H. (1982a) Plasma progesterone levels in the pregnant and non-pregnant tammar, *Macropus eugenii*. *Journal of Endocrinology*, **93**, 99–107.

Hinds, L.A. and Tyndale-Biscoe, C.H. (1982b) Prolactin in the marsupial, *Macropus eugenii*,

during the estrous cycle, pregnancy and lactation. *Biology of Reproduction*, **26**, 391–398.

Hinds, L.A. and Tyndale-Biscoe, C.H. (1985) Seasonal and circadian patterns of circulating prolactin during lactation and seasonal quiescence in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **74**, 173–183.

Hinds, L.A., Evans, S.M. and Tyndale-Biscoe, C.H. (1983) *In vitro* secretion of progesterone by the corpus luteum of the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **67**, 57–63.

Hinds, L.A., Poole, W.E., Tyndale-Biscoe, C.H., van Oorschot, A.H. and Cooper, D.W. (1990a) Reproductive biology and the potential for genetic studies in the tammar wallaby *Macropus eugenii*, in *Mammals from pouches and eggs: Genetics, breeding and evolution of marsupials and monotremes*, (eds J.A.M. Graves, R.M. Hope and D.W. Cooper), *Australian Journal of Zoology*, **37**, 223–34.

Hinds, L., Tyndale-Biscoe, C.H., Shaw, G., Fletcher, T.P. and Renfree, M.B. (1990b) The effects of prostaglandin and prolactin on luteolysis and parturient behaviour in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **88**, 323–333.

Horn, C.A., Fletcher, T.P. and Carpenter, S. (1985) Effects of oestradiol-17β on peripheral plasma concentrations of LH and FSH in ovariectomised tammars (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **73**, 585–592.

Hughes, R.L. (1962) Role of the corpus luteum in marsupial reproduction. *Nature (London)*, **194**, 890–1.

Hughes, R.L. (1965) Comparative morphology of spermatozoa from five marsupial familes. *Australian Journal of Zoology*, **13**, 533–543.

Hughes, R.L. (1974) Morphological studies on implantation in marsupials. *Journal of Reproduction and Fertility*, **39**, 173–186.

Hughes, R.L. (1982) Reproduction in the Tasmanian devil *Sarcophilus harrisii* (Dasyuridae:Marsupialia), in *Carnivorous Marsupials*, (ed. M. Archer), *Royal Zoological Society, New South Wales*, Sydney, Australia, pp. 49–63.

Hughes, R.L. (1984) Structural adaptations of the eggs and the fetal membranes of monotremes and marsupials for respiration and metabolic exchange, in *Respiration and Metabolism of Embryonic Vertebrates*, (ed. R.S. Seymour), Junk, Dordrecht, pp. 389–421.

Hughes, R.L. and Hall, L.S. (1984) Embryonic development in the common brushtail possum *Trichosurus vulpecula*, in *Possums and Gliders*, (eds A.P. Smith and I.D. Hume), Australian Mammal Society, Sydney, pp. 197–212.

Hughes, R.L. and Hall, L.S. (1988) Structural Adaptations of the Newborn Marsupial. In *The Developing Marsupial. Models for Biomedical Research*, Chapter 2 (eds C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin.

Hughes, R.L., Hall, L.S., Tyndale-Biscoe, C.H. and Hinds, L.A. (1989) Evolutionary implications of macropodid organogenesis, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P. Jarman, I.D. Hume and G. Grigg), Surrey Beatty & Sons Pty. Ltd., Sydney. pp. 377–405.

Hutson, J.M., Shaw, G., O, W.S., Short, R.V. and Renfree, M.B. (1988) The ontogeny of Mullerian inhibiting substance production and testicular differentiation, migration and descent in the pouch young of a marsupial. *Development*, **104**, 549–556.

Janssens, P.A. and Hinds, L.A. (1981) Long-term effects of corticosteroid administration in the tammar wallaby (*Macropus eugenii*). *General and Comparative Endocrinology*, **45**, 56–60.

Janssens, P.A. and Ternouth, J.H. (1987) The transition from milk to forage diets. In *The nutrition of herbivores*, (eds. J.B. Hacker and J.H. Ternouth), Academic Press, London, pp. 281–305.

Jurgelski, W. (1971) Administration of test materials to the neonatal North American opossum (*Didelphis marsupialis virginiana* Kerr). *Laboratory Animal Science*, **21**, 748–751.

Kirsch, J.A.W. (1977) The six-percent solution: second thoughts on the adaptedness of the Marsupialia. *American Scientist*, **65**, 276–288.

Kirsch, J.A.W. and Poole, W.E. (1972) Taxonomy and distribution of the grey kangaroos, *Macropus giganteus* Shaw and *Macropus fuliginosus* (Desmarest), and their subspecies (Marsupialia:Macropodidae). *Australian Journal of Zoology*, **20**, 315–339.

Krause, W.J. and Cutts, J.H. (1983) Ultrastructural observations on the shell membrane of the North American opossum (*Didelphis virginiana*). *Anatomical Record*, **207**, 335–338.

Krause, W.J. and Cutts, J.H. (1984) Scanning electron microscopic observations on the opossum yolk sac chorion immediately prior to uterine attachment. *Journal of Anatomy*, **138**, 189–191.

Krause, W.J. and Cutts, J.H. (1985) Placentation

in the opossum, *Didelphis virginiana*. *Acta Anatomica*, **123**, 156–171.

Krause, W.J. and Leeson, C.R. (1973) The postnatal development of the respiratory system of the opossum. I. Light and scanning electron microscopy. *American Journal of Anatomy*, **137**, 337–356.

Krause, W.J., Cutts, J.H. and Leeson, C.R. (1976) Type II pulmonary epithelial cells of the newborn opossum lung. *American Journal of Anatomy*, **146**, 181–188.

Krause, W.J., Cutts, J.H. and Leeson, C.R. (1979a) Morphological observations on the mesonephros in the postnatal opossum, *Didelphis virginiana*. *Journal of Anatomy*, **129**, 377–397.

Krause, W.J., Cutts, J.H. and Leeson, C.R. (1979b) Morphological observations on the metanephros in the postnatal opossum, *Didelphis virginiana*. *Journal of Anatomy*, **129**, 459–477.

Leatherland, J.F. and Renfree, M.B. (1982) Ultrastructure of the non-granulated cells and morphology of the extracellular spaces in the pars distalis of adult and pouch-young tammar wallabies (*Macropus eugenii*). *Cell and Tissue Research*, **227**, 439–450.

Leatherland, J.F. and Renfree, M.B. (1983) Structure of the pars distalis in pouch-young tammar wallabies (*Macropus eugenii*). *Cell and Tissue Research*, **230**, 587–603.

Lee, A.K. and Cockburn, A. (1985) *The Evolutionary Ecology of Marsupials*, Cambridge University Press.

Lemon, M. (1972) Peripheral plasma progesterone during pregnancy and the oestrous cycle in the tammar wallaby *Macropus eugenii*. *Journal of Endocrinology*, **55**, 63–71.

Lemon, M. and Bailey, L.F. (1966) A specific protein difference in the milk from two mammary glands of a red kangaroo. *Australian Jouranl of Experimental Biology and medical Science*, **44**, 703–708.

Lemon, M. and Barker, S. (1967) Changes in milk composition of the red kangaroo, *Megaleia rufa* (Desmarest), during lactation. *Australian Journal of Experimental Biology and Medical Science*, **45**, 213–219.

Lemon, M. and Poole, W.E. (1969) Specific proteins in the whey from milk of the grey kangaroo. *Australian Journal of Experimental Biology and Medical Science*, **47**, 283–285.

Lewis, P.R., Fletcher, T.P. and Renfree, M.B. (1986) Prostaglandin in the peripheral plasma of tammar wallabies during parturition. *Journal of Endocrinology*, **111**, 103–109.

Liggins, G.C. (1982) The fetus and birth, in *Reproduction in Mammals*, 2nd edn, Book No. 2, Embryonic and fetal development, (eds C.R. Austin and R.V. Short), Cambridge University Press, pp. 114–141.

Lillegraven, J.A., Kielan-Jaworowska, Z. and Clemens, W.A. (eds) (1979) *Mesozoic Mammals: The First Two-thirds of Mammalian History*, Chapter 13, University of California Press, Berkeley, pp. 259–276.

Lillegraven, J.A., Thompson, S.D., McNab, B.K. and Patton, J.L. (1987) The origin of eutherian mammals. *Biological Journal of the Linnean Society*, **32**, 281–336.

Lincoln, D.W. and Paisley, A.C. (1982) Neuroendocrine control of milk ejection. *Journal of Reproduction and Fertility*, **65**, 213–226.

Lincoln, D.W. and Renfree, M.B. (1981a) Milk ejection in a marsupial, *Macropus agilis*. *Nature*, **289**, 504–506.

Lincoln, D.W. and Renfree, M.B. (1981b) Mammary gland growth and milk ejection in the agile wallaby *Macropus agilis*, displaying concurrent asynchronous lactation. *Journal of Reproduction and Fertility*, **63**, 193–203.

Lindner, H.R. and Sharman, G.B. (1966) The pregnancy hormone in the red kangaroo (*Megaleia rufa* Desm.). *Second International Congress on Hormonal Steroids*, Excerpta Medica, Amsterdam, p. 371.

Loudon, A.S.I. and Curlewis, J.D. (1989) Evidence that the seasonally breeding Bennett's wallaby *(Macropus rufogriseus rufogriseus)* does not exhibit short-day photorefractoriness. *Journal of Reproduction and Fertilility*, **87**, 641–648.

Loudon, A.S.I., Curlewis, J.D. and English, J. (1985) The effect of melatonin on the seasonal embryonic diapause of the Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Journal of Zoology*, **206**, 35–39.

Loudon, A.S.I., Brinklow, B.R., Gulland, F.D., Boyle, J. and Flint, A.P.F. (1990) The roles of prolactin and the uterus in the control of luteal regression in the Bennett's wallaby *(Macropus rufogriseus rufogriseus)*. *Reproduction Fertility and Development*, **2**, 71–78.

Lyne, A.G. (1974) Gestation period and birth in the marsupial *Isoodon macrourus*. *Australian Journal of Zoology*, **22**, 303–309.

Lyne, A.G. and Hollis, D.E. (1976) Early embryology of the marsupials *Isoodon macrourus* and

Perameles nasuta. *Australian Journal of Zoology*, **25**, 225–231.

Lyne, A.G. and Hollis, D.E. (1979) Observations on the corpus luteum during pregnancy and lactation in the marsupials *Isoodon macrourus* and *Perameles nasuta*. *Australian Journal of Zoology*, **27**, 881–899.

McConnell, S.J. (1984) The pineal gland and reproduction in the tammar wallaby, *Macropus eugenii*. *PhD Thesis*, Australian National University, Canberra.

McConnell, S.J. and Hinds, L.A. (1985) Effect of pinealectomy on plasma melatonin, prolactin and progesterone concentrations during seasonal reproductive aquiescence in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **7**, 433–440.

McConnell, S.J. and Tyndale-Biscoe, C.H. (1985) Response to photoperiod in peripheral plasma melatonin and the effects of exogenous melatonin on seasonal quiescence in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **73**, 529–538.

McConnell, S.J., Tyndale-Biscoe, C.H. and Hinds, L.A. (1986) Change in duration of elevated concentrations of melatonin is the major factor in photoperiod response of the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **77**, 623–632.

McCrady, E. (1938) The embryology of the opossum. *The American Anatomical Memoirs*, **16**, 1–233.

Maule Walker, F.M. (1984) Exocrine and endocrine secretions of the mammary gland: local control in ruminants. *Symposium of the Zoological Society (London)*, **51**, 171–191.

Maynes, G.M. (1973) Reproduction in the parma wallaby, *Macropus parma* Waterhouse. *Australian Journal of Zoology*, **21**, 331–51.

Medawar, P.B. (1953) Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symposioum of the Society of Experimental Biology*, **7**, 328–338.

Merchant, J.C. (1979) The effect of pregnancy on the interval between one oestrus and the next in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **56**, 459–463.

Merchant, J.C. and Calaby, J.H. (1981) Reproductive biology of the red-necked wallaby (*Macropus rufogriseus banksianus*) and Bennett's wallaby (*M.r. rufogriseus*) in captivity. *Journal of Zoology (London)*, **194**, 203–207.

Merchant, J.C. and Sharman, G.B. (1966) Observations on the attachment of marsupial pouch young to the teats and on the rearing of pouch young by foster-mothers of the same or different species. *Australian Journal of Zoology*, **14**, 593–609.

Messer, M. and Green, B. (1979) Milk carbohydrates of marsupials. II. Quantitative and qualitative changes in milk carbohydrates during lactation in the tammar wallaby (*Macropus eugenii*). *Australian Journal of Biological Science*, **32**, 519–531.

Moore, G.P.M. (1978) Embryonic diapause in the marsupial *Macropus eugenii*. Stimulation of nuclear RNA polymerase activity in the blastocyst during resumption of development. *Journal of Cellular Physiology*, **94**, 31–36.

Moors, P.J. (1974) The foeto-maternal relationship and its significance in marsupial reproduction: a unifying hypothesis. *Australian Mammalogy*, **1**, 263–266.

Morgan J. (1829) Description of the mammary organs of the kangaroo. *Transactions of the Linnean Society of London*, **16**, 61–84.

Mori, M., Vigh, S., Miyata, A., Yoshihara, T., Oka, S. and Arimura, A. (1990) Oxytocin is the major prolactin releasing factor in the posterior pituitary. *Endocrinology*, **126**, 1009–1013.

Nicholas, K.R. (1988a) Control of milk protein synthesis in the marsupial *Macropus eugenii*: a model system to study prolactin-dependent development, in *The Developing Marsupial. Models for Biomedical Research*, Chapter 6 (eds. C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin.

Nicholas, K.R. (1988b) Asynchronous dual lactation in a marsupial, the tammar wallaby (*Macropus eugenii*). *Biochemical and Biophysical Research Communication*, **154**, 529–536.

Nicholas, K.R. and Tyndale-Biscoe, C.H. (1985) Prolactin-dependent accumulation of α-lactalbumin in mammary gland explants from the pregnant tammar (*Macropus eugenii*). *Journal of Endocrinology*, **106**, 337–342.

Nicholas, K.R., Messer, M., Elliott, C., Maher, F. and Shaw, D.C. (1987) A novel whey protein synthesized only in late lactation by the mammary gland from the tammar (*Macropus eugenii*). *Biochemical Journal*, **241**, 899–904.

O, W.S., Short, R.V., Renfree, M.B. and Shaw, G. (1988) Primary genetic control of somatic sexual differentiation in a mammal. *Nature (London)*, **331**, 716–717.

O'Donoghue, C.H. (1911) The growth changes in

the mammary apparatus of *Dasyurus* and the relation of the corpora lutea thereto. *Quarterly Journal of Microscopical Science*, **57**, 187–234.

Owen, F.J., Cake, M.H. and Bradshaw, S.D. (1982) Characterization and properties of a progesterone receptor in the uterus of the quokka (*Setonix brachyurus*). *Journal of Endocrinology*, **93**, 17–24.

Owen, R. (1834) On the generation of the marsupial animals, with a description of the impregnated uterus of the kangaroo. *Philosophical Transacations – Royal Society (London)*, **B**, 333–364.

Padykula, H.A. and Taylor, J.M. (1976) Ultrastructural evidence for loss of the trophoblastic layer in the chorioallantoic placenta of Australian bandicoots (Marsupialia:Peramelidae). *Anatomical Record*, **186**, 357–385.

Padykula, H.A. and Taylor, J.M. (1977) Uniqueness of the bandicoot chorioallantoic placenta (Marsupialia:Peramelidae). Cytological and evolutionary interpretations, in *Reproduction and Evolution*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), Australian Academy of Science, Canberra. pp. 303–23.

Padykula, H.A. and Taylor, J.M. (1982) Marsupial placentation and its evolutionary significance. *Journal of Reproduction and Fertility*, Supplement **31**, 95–104.

Panyaniti, W., Carpenter, S.M. and Tyndale-Biscoe, C.H. (1985) Effects of hypophysectomy on folliculogenesis in the tammar *Macropus eugenii* (Marsupialia:Macropodidae). *Australian Journal of Zoology*, **33**, 303–311.

Peaker, M. and Wilde, C. (1987) Milk secretion: Autocrine control. *News in Physiological Sciences*, **2**, 124–126.

Perret, M. and Atramentowicz, M. (1989) Plasma concentrations of progesterone and testosterone in captive woolly opossums (*Caluromys philander*). *Journal of Reproduction and Fertility*, **85**, 31–41.

Pike, I.L. (1981) Comparative studies of embryo metabolism in early pregnancy. *Journal of Reproduction and Fertility*, Supplement **29**, 203–213.

Pilton, P.E. and Sharman, G.B. (1962) Reproduction in the marsupial *Trichosurus vulpecula*. *Journal of Endocrinology*, **25**, 119–136.

Poole, W.E. (1975) Reproduction in the two species of grey kangaroos, *Macropus giganteus* Shaw, and *M. fuliginosus* (Desmarest). II. Gestation, parturition and pouch life. *Australian Journal of Zoology*, **23**, 333–353.

Poole, W.E. and Catling, P.C. (1974) Reproduction in the two species of grey kangaroo, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest). I. Sexual maturity and oestrus. *Australian Journal of Zoology*, **22**, 277–302.

Randall, D., Gannon, B., Runciman, S. and Baudinette, R.V. (1984) Gas transfer by the neonate in the pouch of the tammar wallaby, *Macropus eugenii*, in *Respiration and metabolism of embryonic vertebrates*, (ed. R.S. Seymour), Junk, Dordrecht, pp. 423–436.

Renfree, M.B. (1972a) Influence of the embryo on the marsupial uterus. *Nature*, **240**, 475–477.

Renfree, M.B. (1972b) Embryo–maternal relationships in the tammar wallaby, *Macropus eugenii*. *PhD Thesis*, Australian National University, Canberra.

Renfree, M.B. (1973a) The composition of fetal fluids of the marsupial *Macropus eugenii*. *Developmental Biology*, **33**, 62–79.

Renfree, M.B. (1973b) Proteins in the uterine secretions of the marsupial *Macropus eugenii*. *Developmental Biology*, **32**, 41–49.

Renfree, M.B. (1974) Ovariectomy during gestation in the American opossum, *Didelphis marsupialis virginiana*. *Journal of Reproduction and Fertility*, **39**, 127–130.

Renfree, M.B. (1975) Uterine proteins in the marsupial, *Didelphis marsupialis virginiana*, during gestation. *Journal of Reproduction and Fertility*, **42**, 163–166.

Renfree, M.B. (1977) Feto-placental influences in marsupial gestation, in *Reproduction and Evolution*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), *Australian Academy of Science*, pp. 325–331.

Renfree, M.B. (1978) Embryonic diapause in mammals – a developmental strategy. In *Dormancy and Developmental Arrest: Experimental Analysis in Plants and Animals*, (ed. M.E. Clutter), Academic Press, New York, pp. 1–46.

Renfree, M.B. (1979) Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial. *Nature (London)*, **278**, 549–551.

Renfree, M.B. (1980a) Embryonic diapause in the honey possum *Tarsipes spencerae*. *Search*, **11**, 81.

Renfree, M.B. (1980b) Placental function and embryonic development in marsupials. In *Comparative Physiology: Primitive Mammals*, (eds K. Schmidt-Nielsen, L. Bolis and C.R. Taylor), Cambridge University Press, pp. 269–84.

Renfree, M.B. (1980c) Endocrine activity in mar-

supial placentation, in *Endocrinology 1980*, (eds I.A. Cumming, J.W. Funder and F.A.O. Mendelsohn), Australian Academy of Science, Canberra, pp. 83–86.

Renfree, M.B. (1981a) Embryonic diapause in marsupials. *Journal of Reproduction and Fertility*, Supplement **29**, 67–78.

Renfree, M.B. (1981b) Marsupials: alternative mammals. *Nature (London)*, **293**, 100–101.

Renfree, M.B. (1982) Implantation and placentation, in *Reproduction in Mammals*, 2nd edn, Book No. 2 (eds C.R. Austin and R.V. Short), *Embryonic and Fetal Development*, Cambridge University Press, pp. 26–69.

Renfree, M.B. (1983) Marsupial reproduction: the choice between placentation and lactation, in *Oxford Reviews of Reproductive Biology 5*, (ed. C.A. Finn), Oxford University Press.

Renfree, M.B. (1992) The role of genes and hormones in marsupial sexual differentiation. *Journal of Zoology (London)*, **226**, pp. 165–173.

Renfree, M.B. (1993) Ontogeny, genetic control and phylogeny of female reproduction in monotreme and therian mammals. In *Mammal Phylogeny*. (eds F.S. Szalay, M.J. Novacek and M.C. McKenna), Springer-Verlag, New York, pp. 4–20.

Renfree, M.B. (1993) Sexual dimorphisms in the gonads and reproductive tract of marsupial mammals. In *The Differences between the Sexes*. (eds R.V. Short and E. Balban), Cambridge University Press, (in press).

Renfree, M.B. and Calaby, J.H. (1981) Background to delayed implantation and embryonic diapause. *Journal of Reproduction and Fertility*, Supplement **29**, 1–9.

Renfree, M.B. and Heap, R.B. (1977) Steroid metabolism by the placenta, corpus luteum and endometrium during pregnancy in the marsupial, *Macropus eugenii*. *Theriogenology*, **8**, 164.

Renfree, M.B. and Short, R.V. (1984) Seasonal reproduction in marsupials, in *Proceedings of the 7th International Congress of Endocrinology*, (eds F. Labrie and L Proulx), Excerpta Medica International Congress Series, Elsevier, Amsterdam, pp. 789–92.

Renfree, M.B. and Short, R.V. (1988) Sex determination in marsupials: evidence for a marsupial-eutherian dichotomy. *Philosophical Transactions – Royal Society (London)*, **B322**, 41–53.

Renfree, M.B. and Tyndale-Biscoe, C.H. (1973) Intra-uterine development after diapause in the marsupial *Macropus eugenii*. *Developmental Biology*, **32**, 28–40.

Renfree, M.B. and Tyndale-Biscoe, C.H. (1978) Manipulation of marsupial embryos and pouch young, in *Methods in Mammalian Reproduction*, Chapter 15 (ed. J.C. Daniel), Academic Press, New York, pp. 307–331.

Renfree, M.B. and Young, I.R. (1979) Steroids in pregnancy and parturition in the marsupial, *Macropus eugenii*. *Journal of Steroid Biochemistry*, **11**, 515–522.

Renfree, M.B., Green, S.W., and Young, I.R. (1979) Growth of the corpus luteum and its progesterone content during pregnancy in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **57**, 131–136.

Renfree, M.B., Lincoln, D.W., Almeida, O.F.X. and Short, R.V. (1981) Abolition of seasonal embryonic diapause in a wallaby by pineal denervation. *Nature (London)*, **293**, 138–139.

Renfree, M.B., Wallace, G.I. and Young, I.R. (1982) Effects of progesterone, oestradiol-17β and androstenedione on follicular growth after removal of the corpus luteum during lactational and seasonal quiescence in the tammar wallaby. *Journal of Endocrinology*, **92**, 397–403.

Renfree, M.B., Russell, E.M. and Wooller, R.D. (1984a) Reproduction and life history of the honey possum, *Tarsipes rostratus*, in *Possums and Gliders*, (eds A.P. Smith and I.D. Hume), Australian Mammal Society, Sydney, pp. 427–37.

Renfree, M.B., Flint, A.P.F., Green, S.W. and Heap, R.B. (1984b) Ovarian steroid metabolism and luteal oestrogens in the corpus luteum of the tammar wallaby. *Journal of Endocrinology*, **101**, 231–240.

Renfree, M.B., Shaw, G. and Short, R.V. (1987) Sexual differentiation in marsupials. In *NIH Workshop on Genetic Markers of Sex Differentiation*, (eds F.P. Haseltine, M.E. McClure and E.H. Goldberg), Plenum Press, New York, pp. 27–41.

Renfree, M.B., Fletcher, T.P., Blanden, D.R., Lewis, P.R., Shaw, G., Gordon, K., Short, R.V., Parer-Cook, E. and Parer, D. (1989) Physiological and behavioural events around the time of birth in macropodid marsupials, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P. Jarman, I.D. Hume and G. Grigg), Surrey Beatty & Sons Pty. Ltd., Sydney, pp. 323–337.

Renfree, M.B., Robinson, E.S., Short, R.V. and VandeBerg, J.L. (1990) Mammary glands in male marsupials. 1. Primordia in neonatal opossums,

Didelphis virginiana and *Monodelphis domestica*. *Development*, **110**, 385–390.

Renfree, M.B., Wilson, J.D., Short, R.V., Shaw, G. and George, F.W. (1992) Steroid hormone content of the gonads of the tammar wallaby during sexual differentiation. *Biology of Reproduction*, **47**, 644–647.

Risman, G.C. (1947) The effects of oestradiol and progesterone on the reproductive tract of the opossum and their possible relation to parturition. *Journal of Morphology*, **81**, 343–397.

Robinson, E.S., Renfree, M.B., Short, R.V. and VandeBerg, J.L. (1991) Mammary glands in male marsupials. 2. Development and regression of mammary primordia in *Monodelphis domestica* and *Didelphis virginiana*. *Reproduction Fertility and Development*, **3**, 295–301.

Rodger, J.C. (1990) Prospects for the artificial manipulation of marsupial reproduction, in *Mammals from pouches and eggs: Genetics, breeding and evolution of marsupials and monotremes*. (eds J.A.M. Graves, R.M. Hope and D.W. Cooper), *Australian Journal of Zoology*, **37**, 249–258.

Rodger, J.C. and Bedford, J.M. (1982a) Separation of sperm pairs and sperm–egg interaction in the opossum, *Didelphis virginiana*. *Journal of Reproduction and Fertility*, **64**, 171–179.

Rodger, J.C. and Bedford, J.M. (1982b) Induction of oestrus, recovery of gametes, and the timing of fertilization events in the opossum, *Didelphis virginiana*. *Journal of Reproduction and Fertility*, **64**, 159–169.

Rose, R.W. (1986) The control of pouch vacation in the Tasmanian bettong, *Bettongia gaimardi*. *Australian Journal of Zoology*, **34**, 485–491.

Rose, R.W. (1987) Reproductive energetics of two Tasmanian rat-kangaroos (Potoroinae:Marsupialia), *Symposium of the Zoological Society (London)*, **57**, 149–165.

Rose, R.W. and McCartney, D.J. (1982) Reproduction of the red-bellied pademelon, *Thylogale billardierii* (Marsupialia). *Australian Wildlife Research*, **9**, 27–32.

Russell, E.M. (1982a). Patterns of parental care and parental investment in marsupials. *Biological Reviews*, **57**, 423–486.

Russell, E.M. (1982b) Parental investment and desertion of young in marsupials. *American Naturalist*, **119**, 744–748.

Russell, E.M. (1989) Maternal behaviour in the Macropodoidea, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P. Jarman, I.D. Hume and G. Grigg), Surrey Beatty & Sons, Pty. Ltd., Sydney, pp. 549–469.

Sadleir, R.M.F.S. and Tyndale-Biscoe, C.H. (1977) Photoperiod and the termination of embryonic diapause in the marsupial *Macropus eugenii*. *Biology of Reproduction*, **16**, 605–608.

Schallenberger, E., Richardson, D.W. and Knobil, E. (1981) Role of prolactin in the lactational amenorrhoea of the rhesus monkey (*Macaca mulatta*). *Biology of Reproduction*, **25**, 370–374.

Seiler, B.W. (1828) Einige Bemerkungen uber die arste Geburt des Kangeru-Embryo und seine Ernahrung in dem Beutel. *Isis von Oken*, **12**, 475–477.

Selwood, L. (1980) A timetable of embryonic development of the dasyurid marsupial *Antechinus stuartii* (Macleay). *Australian Journal of Zoology*, **28**, 649–668.

Selwood, L. (1981) Delayed embryonic development in the dasyurid marsupial *Antechinus stuartii*. *Journal of Reproduction and Fertility*, Supplement **29**, 79–82.

Selwood, L. (1982) A review of maturation and fertilization in marsupials with special reference to the dasyurid: *Antechinus stuartii*, in *Carnivorous Marsupials*, (ed. M. Archer), Royal Zoological Society of New South Wales, Sydney, pp. 65–76.

Selwood, L. (1986) Cleavage *in vitro* following destruction of some blastomeres in the marsupial *Antechinus stuartii* (Macleay). *Journal of Embryology and Experimental Morphology*, **92**, 71–84.

Selwood, L. (1987) Embryonic development in culture of two Dasyurid marsupials, *Sminthopsis crassicaudata* (Gould) and *Sminthopsis macroura* (Spencer), during cleavage and blastocyst formation. *Gamete Research*, **16**, 355–370.

Selwood, L. (1992) Mechanisms underlying the development of pattern in marsupial embryos. *Current Topics in Developmental Biology*, **27**, 175–233.

Selwood, L. and Sathananthan, A.H. (1988) Ultrastructure of early cleavage and yolk extrusion in the marsupials *Antechinus stuartii*. *Journal of Morphology*, **195**, 327–44.

Selwood, L. and Young, G.J. (1983) Cleavage *in vivo* and in culture in the dasyurid marsupial *Antechinus stuartii* (Macleay). *Journal of Morphology*, **176**, 43–60.

Sernia, C. and Tyndale-Biscoe, C.H. (1979) Prolactin receptors in the mammary gland, corpus luteum and other tissues of the tammar

wallaby, *Macropus eugenii*. *Journal of Endocrinology*, **83**, 79–89.

Sernia, C., Hinds, L. and Tyndale-Biscoe, C.H. (1980) Progesterone metabolism during embryonic diapause in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **60**, 139–47.

Sharman, G.B. (1955a). Studies on marsupial reproduction. II. The oestrous cycle of *Setonix brachyurus*. *Australian Journal of Zoology*, **3**, 44–55.

Sharman, G.B. (1955b) Studies on marsupial reproduction. III. Normal and delayed pregnancy in *Setonix brachyurus*. *Australian Journal of Zoology*, **3**, 56–70.

Sharman, G.B. (1961) The embryonic membranes and placentation in five genera of diprotodont marsupials. *Proceedings of the Zoological Society of London*, **137**, 197–220.

Sharman, G.B. (1962) The initiation and maintenance of lactation in the marsupial, *Trichosurus vulpecula*. *Journal of Endocrinology*, **25**, 375–385.

Sharman, G.B. (1965a) The effects of the suckling stimulus and oxytocin injection on the corpus luteum of delayed implantation in the red kangaroo. *Excerpta Medica*, **83**, 669–674.

Sharman, G.B. (1965b) The effects of suckling on normal and delayed cycles of reproduction in the red kangaroo. *Sonderdruck aus Zeitschrift für Saugetierkunde*, **30**, 10–20.

Sharman, G.B. (1970) Reproductive physiology of marsupials. *Science (New York)*, **167**, 1221–1228.

Sharman, G.B. (1973) Adaptations of marsupial pouch young for extra-uterine existence. In *The Mammalian Fetus In Vitro*, (ed. C.R. Austin), Chapman & Hall, London, pp. 67–90.

Sharman, G.B. and Berger, P.J. (1969) Embryonic diapause in marsupials. *Advances in Reproductive Physiology*, **4**, 211–240.

Sharman, G.B. and Calaby, J.H. (1964) Reproductive behaviour in the red kangaroo, *Megaleia rufa*, in captivity. *CSIRO Wildlife Research*, **9**, 58–85.

Sharman, G.B., Hughes, R.L. and Cooper, D.W. (1990) The chromosomal basis of sex differentiation in marsupials, in *Mammals from pouches and eggs: Genetics, breeding and evolution of marsupials and monotremes*, (eds J.A.M. Graves, R.M. Hope and D.W. Cooper), *Australian Journal of Zoology*, **37**, 451–466.

Shaw, G. (1983a) Effect of $PGF_{2\alpha}$ on uterine activity, and concentrations of 13,14–dihydro–15–keto–$PGF_{2\alpha}$ in peripheral plasma during parturition in the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **69**, 429–436.

Shaw, G. (1983b) Pregnancy after diapause in the tammar wallaby, *Macropus eugenii*. *PhD Thesis*, Murdoch University, Western Australia.

Shaw, G. (1990) Control of parturient behaviour by prostaglandin $F_{2\alpha}$in the tammar wallaby *(Macropus eugenii)*. *Journal of Reproduction and Fertility*, **88**, 335–342.

Shaw, G. and Renfree, M.B. (1984) Concentrations of oestradiol-17β in plasma and corpora lutea throughout pregnancy in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **72**, 29–37.

Shaw, G. and Renfree, M.B. (1986). Uterine and embryonic metabolism after diapause in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **76**, 339–347.

Shaw, G. and Rose, R.W. (1979) Delayed gestation in the potoroo *Potorous tridactylus* (Kerr). *Australian Journal of Zoology*, **27**, 901–912.

Shaw, G., Renfree, M.B., Short, R.V. and O, W.S. (1988) Experimental manipulation of sexual differentiation in wallaby pouch young with exogenous steroids. *Development*, **104**, 689–701.

Shaw, G., Renfree, M.B. and Short, R.V. (1990) Primary genetic control of sexual differentiation in marsupials. *Australian Journal of Zoology*, **37**, 443–450.

Shaw, G., Fletcher, T.P. and Renfree, M.B. (1992) Corticosteroids induce early parturition in a marsupial. *Journal of Reproduction and Fertility*, Abstract Series no. **9**, p.135.

Shorey, C.D. (1968) An electron microscopy study of pituitary–ovarian changes during oogenesis and embryonic development in *Trichosurus vulpecula*. *MSc Thesis*, University of New South Wales, Sydney, Australia.

Shorey, C.D. and Hughes, R.L. (1973a) Development, function and regression of the corpus luteum in the marsupial *Trichosurus vulpecula*. *Australian Journal of Zoology*, **21**, 477–489.

Shorey, C.D. and Hughes, R.L. (1973b) Cyclical changes in the uterine endometrium and peripheral plasma concentrations of progesterone in the marsupial *Trichosurus vulpecula*. *Australian Journal of Zoology*, **21**, 1–19.

Shorey, C.D. and Hughes, R.L. (1975) Uterine response to ovariectomy during the proliferative and luteal phases in the marsupial, *Trichosurus vulpecula*. *Journal of Reproduction and Fertility*, **42**, 221–228.

Short, R.V., Flint, A.P.F. and Renfree, M.B. (1985)

Influence of passive immunization against GnRH on pregnancy and parturition in the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **75**, 567–575.

Short, R.V., Renfree, M.B. and Shaw, G. (1988) Sexual development in marsupial pouch young, in *Development of Young Marsupials: Models for Biomedical Research*, (eds C.H. Tyndale-Biscoe and P. Janssens), Springer-Verlag, Berlin, pp. 200–210.

Smith, J.G., Hanks, J. and Short, R.V. (1969) Biochemical observations on the corpora lutea of the African elephant, *Loxodonta africana*. *Journal of Reproduction and Fertility*, **20**, 111–117

Smith, M.J. (1981) Morphological observations on the diapausing blastocyst of some macropodid marsupials. *Journal of Reproduction and Fertility*, **61**, 483–446.

Smith, M.J. (1989) Release of embryonic diapause in the brush-tailed bettong, *Bettongia penicillata*, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds P. Jarman, I.D. Hume and G. Grigg), Surrey Beatty & Sons Pty. Ltd., Sydney, pp. 317–321.

Stewart, F. (1984) Mammogenesis and changing prolactin receptor concentrations in the mammary glands of the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **71**, 141–148.

Stewart, F. and Tyndale-Biscoe, C.H. (1982) Prolactin and luteinizing hormone receptors in marsupial corpora lutea: relationship to control of luteal function. *Journal of Endocrinology*, **92**, 63–72.

Stewart, F. and Tyndale-Biscoe, C.H. (1983) Pregnancy and parturition in marsupials. *Current Topics in Experimental Endocrinology*, **4**, pp. 1–33.

Stewart, G.M., Addey, C.V.P., Knight, C.H. and Wilde, C.J. (1988) Autocrine regulation of casein turnover in goat mammary explants. *Journal of Endocrinology*, **118**, R1–R3.

Sutherland, R.L., Evans, S.M. and Tyndale-Biscoe, C.H. (1980) Macropodid marsupial luteinising hormone: validation of assay procedures and changes in plasma levels during the oestrous cycle in the female tammar wallaby (*Macropus eugenii*). *Journal of Endocrinology*, **86**, 1–12.

Thorburn, G.D., Cox, R.I. and Shorey, C.D. (1971) Ovarian steroid secretion rates in the marsupial *Trichosurus vulpecula*. *Journal of Reproduction and Fertility*, **24**, 139.

Thorburn, G.D., Hooper, S.B., Rice, G.E. and Fowden, A.L. (1988) Luteal regression and parturition: A comparison, in *The Onset of Labour: Cellular and Integrative Mechanisms*, Eds. D. McNellis, J. Challis, P. MacDonald, P. Nathanielsz and J. Roberts), Perinatology Press, New York, pp. 185–210.

Thornber, E.J., Renfree, M.B. and Wallace, G.I. (1981) Biochemical studies of intrauterine components of the tammar wallaby *Macropus eugenii* during pregnancy. *Journal of Embryology and Experimental Morphology*, **62**, 325–338.

Towers, P.A., Shaw, G. and Renfree, M.B. (1986) Urogenital vasculature and local steroid concentrations in the uterine branch of the ovarian vein of the female tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **78**, 37–47.

Tyndale-Biscoe, C.H. (1963a) Effects of ovariectomy in the marsupial, *Setonix brachyurus*. *Journal of Reproduction and Fertility*, **6**, 25–40.

Tyndale-Biscoe, C.H. (1963b) The role of the corpus luteum in the delayed implantation in marsupials, in *Delayed Implantation*, (ed. A.C. Enders), Chicago University Press, pp. 15–32.

Tyndale-Biscoe, C.H. (1966) The marsupial birth canal. *Symposium of the Zoological Society (London)*, **15**, 233–250.

Tyndale-Biscoe, C.H. (1968) Reproduction and post-natal development in the marsupial *Bettongia lesueur* (Quoy and Gaimard). *Australian Journal of Zoology*, **16**, 577–602.

Tyndale-Biscoe, C.H. (1969) Relaxin activity during the oestrous cycle of the marsupial, *Trichosurus vulpecula* (Kerr). *Journal of Reproduction and Fertility*, **19**, 191–193.

Tyndale-Biscoe, C.H. (1970) Resumption of development by quiescent blastocysts transferred to primed, ovariectomised recipients in the marsupial, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **23**, 25–32.

Tyndale-Biscoe, C.H. (1973) *Life of Marsupials*, Arnold, London.

Tyndale-Biscoe, C.H. (1979) Hormonal control of embryonic diapause and reactivation in the tammar wallaby, in *Maternal Recognition of Pregnancy*, Ciba Foundation Symposium 64 (new series), *Excerpta Medica (Amsterdam)*, pp. 173–190.

Tyndale-Biscoe, C.H. (1981) Evidence for relaxin in marsupials, in *Relaxin*, (ed. G. Bryant Greenwood, H. Niall and F. Greenwood), North Holland/Elsevier Biomedical Press, Amsterdam, pp. 225–232.

Tyndale-Biscoe, C.H. (1984) Mammals-Marsupials, in *Marshall's Physiology of Repro-*

duction, 4th edn, Vol. 1, Chapter 5, (ed. G.E. Lamming), Churchill Livingstone, Edinburgh, pp. 386–454.

Tyndale-Biscoe, C.H. (1986) Embryonic diapause in a marsupial: roles of the corpus luteum and pituitary in its control, in *Comparative Endocrinology: Developments and Directions*, Alan R. Liss, New York, pp. 137–155.

Tyndale-Biscoe, C.H. (1989) The adaptiveness of reproductive processes, in *Kangaroos, Wallabies and Rat Kangaroos*, (eds G. Grigg, P. Jarman and I. Hume), Surrey Beatty & Sons Pty. Ltd., Sydney, pp. 277–285.

Tyndale-Biscoe, C.H. and Hawkins, J. (1977) The corpora lutea of marsupials, aspects of function and control, in *Reproduction and Evolution*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), Australian Academy of Science, Canberra, pp. 245–252.

Tyndale-Biscoe, C.H. and Hearn, J.P. (1981) Pituitary and ovarian factors associated with seasonal quiescence of the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **63**, 225–230.

Tyndale-Biscoe, C.H. and Hinds, L.A. (1984) Seasonal patterns of circulating progesterone and prolactin and response to bromocriptine in the female tammar, *Macropus eugenii*. *General and Comparative Endocrinology*, **53**, 58–68.

Tyndale-Biscoe, C.H. and Hinds, L.A. (1989) The hormonal milieu during early development in marsupials, in *Development of Preimplantation Embryos and Their Environment*, (ed. K. Yoshinaga), Alan R. Liss, New York, pp. 237–246.

Tyndale-Biscoe, C.H. and Janssens, P.A. (1988) Chapter I, *The Developing Marsupial. Models for Biomedical Research*, Springer-Verlag, Berlin.

Tyndale-Biscoe, C.H. and Renfree, M.B. (1987) *Reproductive Physiology of Marsupials*. Cambridge University Press.

Tyndale-Biscoe, C.H. and Rodger J.C. (1978) Differential transport of spermatozoa into the two sides of the genital tract of a monovular marsupial, the tammar wallaby *Macropus eugenii*. *Journal of Reproduction and Fertility*, **52**, 37–43.

Tyndale-Biscoe, C.H., Hearn, J.P. and Renfree, M.B. (1974) Control of reproduction in macropodid marsupials. *Journal of Endocrinology*, **63**, 589–614.

Tyndale-Biscoe, C.H., Hinds, L.A., Horn, C.A. and Jenkin, G. (1983) Hormonal changes at oestrus, parturition and post-partum oestrus in the tammar wallaby (*Macropus eugenii*). *Journal of Endocrinology*, **96**, 155–161.

Tyndale-Biscoe, C.H., Stewart, F. and Hinds, L.A. (1984) Some factors in the initiation and control of lactation in the tammar wallaby. *Symposium of the Zoological Society (London)*, **51**, 389–401.

Tyndale-Biscoe, C.H., Hinds, L.A. and McConnell S.J. (1986) Seasonal breeding in a marsupial: opportunities of a new species for an old problem. *Recent Progress in Hormone Research*, **42**, 471–512.

Tyndale-Biscoe, C.H., Hinds, L.A. and Horn, C.A. (1988) Fetal role in the control of parturition in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **82**, 419–428.

Ullmann, S.L. (1981a) Observations on the primordial germ cells of bandicoots (Peramelidae:Marsupialia). *Journal of Anatomy*, **132**, 581–595.

Ullmann, S.L. (1981b) Sexual differentiation of the gonads in bandicoots (Peramelidae:Marsupialia). *Proceedings of the Vth Workshop on the Development and Function of the Reproductive Organs*. Int. Congr. Ser. 559, (ed. A.G. Byskov and H. Peters), Excerpta Medica, Amsterdam, pp. 41–50.

Ullmann, S.L. (1993) Differentiation of the gonads and initation of mammary gland and scrotum development in the brush-tailed possum, *Trichosurus vulpecula* (Marsupialia). *Anatomy and Embryology*, **187**, 475–484.

Walker, M.T. and Gemmell, R.T. (1983a) Plasma concentrations of progesterone, oestradiol-17β and 13, 14–dihydro–15–oxo–prostaglandin $F_{2\alpha}$ in the pregnant wallaby (*Macropus rufogriseus rufogriseus*). *Journal of Endocrinology*, **97**, 369–377.

Walker, M.T. and Gemmell, R.T. (1983b) Organogenesis of the pituitary, adrenal, and lung at birth in the wallaby, *Macropus rufogriseus*. *American Journal of Anatomy*, **168**, 331–344.

Walker, M.T. and Rose, R. (1981) Prenatal development after diapause in the marsupial *Macropus rufogriseus*. *Australian Journal of Zoology*, **29**, 167–187.

Walker, M.T., Gemmell, R.T. and Hughes, R.L. (1983) Ultrastructure of the reactivated corpus luteum after embryonic diapause in the marsupial, *Macropus rufogriseus banksianus*. *Cell and Tissue Research*, **228**, 61–74.

Wallace, G.I. (1981) Uterine factors and delayed implantation in macropodid marsupials. *Journal of Reproduction and Fertility*, Supplement **29**, 173–181.

Ward, K.L. and Renfree, M.B. (1984) Effects of

progesterone on parturition in the tammar, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **72,** 21–28.

Ward K.L. and Renfree, M.B. (1988) Reproduction in female feathertail gliders, *Acrobates pygmeaus*. *Journal of Zoology (London)*, **216,** 225–240.

Ward, S. (1988) Life histories of the small diprotodont marsupials. *PhD Thesis*, Monash University, Melbourne, Australia.

Wilde, C.J., Calvert, D.T., Daly, A. and Peaker, M. (1987) The effect of goat milk fractions on synthesis of milk constituents by rabbit mammary explants and on milk yield *in vitro*. *Biochemical Journal*, **242,** 285–288.

Wilde, C.J., Addey, C.V.P., Casey, M.J., Blatchford, D.R. and Peaker, M. (1988) Feedback inhibition of milk secretion: The effect of a fraction of goat milk on milk yield and composition. *Quarterly Journal of Experimental Physiology*, **73,** 391–397.

Wilkes, G.E. (1984) The development of structure and function of the kidney in the tammar wallaby *Macropus eugenii*. *PhD Thesis*, Australian National University, Canberra, Australia.

Wilkes, G.E. and Janssens, P.A. (1986a) Development of urine concentrating ability in pouch young of a marsupial the tammar wallaby *(Macropus eugenii)*. *Journal of Comparative Physiology*, **156B,** 573–582.

Wilkes, G.E. and Janssens, P.A. (1986b) Physiological and metabolic changes associated with weaning in the tammar wallaby *Macropus eugenii*. *Journal of Comparative Physiology*, **156B**, 829–837.

Wilkes, G.E. and Janssens, P.A. (1988) The development of renal function, in *The Developing Marsupial. Models for Biomedical Research*, (eds. C.H. Tyndale-Biscoe and P.A. Janssens), Springer-Verlag, Berlin, pp. 176–189.

Williamson, P., Fletcher, T.P. and Renfree, M.B. (1990) Sexual maturity in the male tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility*, **88,** 323–333.

Wooller, R.D., Renfree, M.B., Russell, E.M., Dunning, A., Green, S.W. and Duncan, P. (1981) Seasonal changes in a population of the nectar-feeding marsupial *Tarsipes spencerae* (Marsupialia: Tarsipedidae). *Journal of Zoology (London)*, **195**, 267–279.

Woolley, P. (1966) Reproduction in *Antechinus* spp. and other dasyurid marsupials. *Symposium of the Zoological Society (London)*, **15,** 281–294.

Yadav, M. (1971) The transmission of antibodies across the gut of pouch young marsupials. *Immunology*, **21**, 839–851.

Young, C.E. and McDonald, I.R. (1982) Oestrogen receptors in the genital tract of the Australian marsupial *Trichosurus vulpecula*. *General and Comparative Endocrinology*, **46,** 417–427.

Young, I.R. (1978) The physiology of parturition in the macropodid *Macropus eugenii* (Desmarest). *PhD Thesis*, Murdoch University, Western Australia.

Young, I.R. and Renfree, M.B. (1979) The effects of corpus luteum removal during gestation on parturition in the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **56**, 249–254.

FETAL PHYSIOLOGY 8

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8.1 INTRODUCTION

This chapter is concerned with the physiology of the developing mammal from the time the major organs have developed to birth. Fetal physiology is of interest not only because during fetal life the foundations for future growth and performance are established, but also because the physiology of one animal growing within another is of interest for its own sake. As the fetus grows in size and the various body systems develop, so the 'physiology' of the fetus changes. Much of what we know has been based on studies in the last third of gestation when the fetus is relatively large. Special interest has also been directed to the fetus immediately before birth for, by understanding the behaviour of the fetus before birth, it is hoped to limit the damage caused by the delivery and to optimize the care of the newborn afterwards. Less is known about the physiology of the fetus in mid-gestation, although the development of several clinical techniques (ultrasonography, amniocentesis and fetal blood sampling) over the last decade has increased our knowledge of the biology of the human mid-trimester fetus (Pesonen *et al.*, 1983; Nicolaides *et al.*, 1985; Forestier, 1987; Forestier *et al.*, 1987; Peters and Nicolaides, 1990; Fisk and Rodeck, 1992; Stephenson, 1993).

We begin with a brief description of the intrauterine environment, followed by an outline of intrauterine growth and development, and the nature of the major hurdle the developing mammal has to jump at the time of birth. Section 8.2 gives an outline of the functions of the placenta related to the fetus, section 8.3 describes the working of the major systems before birth and section 8.4 highlights the adjustments made in those systems after birth. The endocrinology of pregnancy is considered elsewhere. Whilst the purpose of most functions is to support or protect the brain, the growth and function of the developing mammalian brain are not considered.

8.1.1 THE INTRAUTERINE ENVIRONMENT

The mammalian fetus has been likened to a fish swimming in its own private pool. Fellows of the Royal Society, soon after its formation, studied the survival of fish out of water in an attempt to understand how the fetus lived in the womb and adjusted after birth (Birch, 1956–57). It was a phenomenon which puzzled W. Harvey (1651). Barcroft (1946), a pioneer of fetal physiology, drew an analogy with a climber up a mountain; others prefer the idea of an astronaut floating in space with a supply cord. Helpful though these analogies are to illustrate some of the unusual aspects of the fetal environment, they can be misleading with respect to the whole.

The uterus protects the new mammal during a critical stage in its development. The membranous sac in which the fetus floats contains water with traces of salts and some protein debris of clinical interest and little else. In man, in the first half of pregnancy amniotic fluid volume related to fetal mass (Lind, 1978) is around 1.8 l per kg fetal body weight, and is probably a dialysate of fetal plasma. In mid-gestation, the skin lays down keratin and other chemicals which make the skin watertight, then the fetal fluid compartments cease to exchange through the skin with the surrounding amniotic fluid. In the second half of human pregnancy, fetal urine is the main contribution to amniotic fluid, the osmolality and sodium and chloride contents fall slightly, and the volume relates more closely to placental mass and is around 0.27 l/kg at term (Lind, 1981). There are more extensive studies on the characteristics and turnover of the fluids that surround other fetal mammals, particularly the sheep (Mellor, 1980).

The temperature of the fluid is determined by the mother's body temperature and, because the fetal metabolic rate is higher than that of the mother, the amniotic fluid tem-

Table 8.1 Gestation, birth weight and placental weight in various mammals.

	Gestation (days)	*Newborn body weight (g)*	*Placenta weight (g)*	*Placenta as a percentage of body weight*
Sheep	147	4500	350	8
Man	**280**	**3500**	**500**	**14**
Pig	120	1300	200	15
Monkey	168	500	100	20
Cat	63	100	15	15
Guinea pig	67	85	5	6
Rabbit	31	50	4	8
Rat	22	5	0.5	11
Mouse	19	2	0.01	9

perature is a fraction of a degree higher than the mother's deep body temperature. When the mother has a fever then the temperature of amniotic fluid rises, as does the fetal body temperature (Abrams, 1978).

The uterus protects the fetus from infection. The surrounding fluid is sterile. The placenta also protects the fetus, to some extent, from infectious organisms that enter the maternal bloodstream, depending on the gestation and the organism. Most common bacteria do not cross the placenta, but in man syphilis and listeria can both affect the fetus. Viraemia leads to fetal infection more commonly than bacteraemia. It has been known for some time that rubella (German measles) virus, varicella zoster virus (chicken pox) (G. Gilbert, 1993; McIntosh and Isaacs, 1993) and cytomegalovirus can all cross the placenta of the human fetus and cause miscarriage or fetal abnormalities. In the case of rubella, about 40% of women with a primary infection in pregnancy will pass the virus on to the fetus (Stagno, 1990), but it is not clear why transplacental infection does not occur in the other 60%. Human immunodeficiency virus and human parvovirus are more recently characterized viruses that can penetrate the defences of the human placenta to infect the fetus. The placenta can also be breached by parasites, such as those which cause toxoplasmosis (Peckham and Logan, 1993) and malaria. The rate of transmission of *Toxoplasma* from mother to fetus increases with increasing gestational age at the time of maternal infection (Holliman, 1992). Toxoplasmosis is one example of cross-species transmission of a zoonosis affecting both mother and fetus (Holliman, 1992).

The intrauterine environment is dark (there is no light to stimulate the eyes) and many mammals are born with fused eyelids. It is not silent: the sound of the heart beats and the bowel borborygmi reach the fetal ears but obviously have no meaning for the fetus. The fetus can contract its muscles and fetal movements do occur and are important for development (Prechtl, 1988), but the fetus is limited by the space available, and the movements become more restricted as the fetus grows. Fetal restraint can distort fetal growth causing postural deformities. In the more severe cases, often associated with profound oligohydramnios (sections 8.3.3b and 8.3.4), permanent abnormalities such as clubfoot and joint contractures may occur (Hensinger and Jones, 1992).

8.1.2 THE LENGTH OF GESTATION AND MATURITY AT BIRTH

Table 8.1 gives the length of gestation of some mammals commonly used for physiological studies and compares them with man.

Table 8.2 Fetal weight as a percentage of maternal body weight.

	Maternal body weight (kg)	*Newborn birth weight (g)*	*Average litter size*	*Fetal weight as a percentage of maternal weight*
Pig	130	1200	8	6.8
Sheep	70	4000	2	11.4
Man	**56**	**3500**	**1**	**5.6**
Monkey (rhesus)	8	500	1	6.3
Rabbit	2.5	50	6	14.0
Guinea pig	0.7	85	3	36.3
Rat	0.15	5	7	23.4
Mouse	0.03	1.5	8	37.3

In general, the larger the mother the longer the gestation. The mouse makes a new being capable of independent existence in 19 days; man takes 9 months. The newborn lamb weighs a little more, on average, than the human newborn but is made in half the time and is able to run and skip soon after birth. Thus, whilst the intrauterine environment is remarkably similar between species, the rate of growth and maturation of the fetus differs as widely as the appearance and performance of the adults. Clearly, inferences about one species based on data obtained from the study of another need to be drawn with care.

Table 8.2 also gives the litter size and an estimate of the total fetal weight as a percentage of maternal body weight.

Again there are wide variations; in the larger mammals the fetal weight forms a lower fraction. In the smaller mammals the fetuses alone (without the uterus, the fetal membranes and fluids) must make considerable demands not only on the mother's physical strength but also on the supplies of oxygen and nutrients. These demands are of a different order, relatively speaking, to the burden that the pregnancy at term places on the larger species.

Comparative physiology is fascinating in this area as in many others. The newborn polar bear weighs about 0.5 kg, which is only 0.3% of the weight of the mother; conversely, a single newborn bat can weigh as much as 30% of the adult weight. Analysis of the relationship between fetal and maternal weight has been the subject of a number of articles (Leitch *et al.*, 1959; Widdowson, 1970; Sacher and Staffeldt, 1974; Hofman, 1983). It has been proposed that the length of gestation is determined, at least in part, by the demands of brain growth and development.

The object of reproduction is to produce offspring capable of independent survival in the environment of their parents. In general, the young fall into two categories: those born into a protected environment, such as a nest or within the fur or arms of the parent, and those who run free soon after the moment of birth. The motor skills of the former are limited and the development of their motor cortex is at an early stage but they are very aware; their sensory cortex is well advanced. By contrast, in those that run free at birth both motor and sensory neural maturation are fairly advanced at birth.

The terms maturity and maturation, whilst clearly relevant to the subject, can be used in a confusing way. If adult size and performance are the gold standards, then all the fetal organs are immature. Obstetricians speak of a mature fetus in comparison with the term infant. However, in terms of biological function, the healthy fetus has physiological responses that meet its requirements at all times and thus to speak of immaturity of function of, for example, the fetal kidney and

Table 8.3 Growth rates before and after birth. (From Widdowson, 1970.)

	Length of gestation (days)	*Fetal growth rate (g/day)*	*Birth weight (kg)*	*Suckling growth rate (g/day)*
Calf	280	125	35	580
Man	**280**	**12.5**	**3.5**	**25**
Pig	120	4.2	1.5	295
Cat	63	1.6	0.1	14
Rat	21	0.42	0.005	1.7
Mouse	21	0.09	0.002	0.47

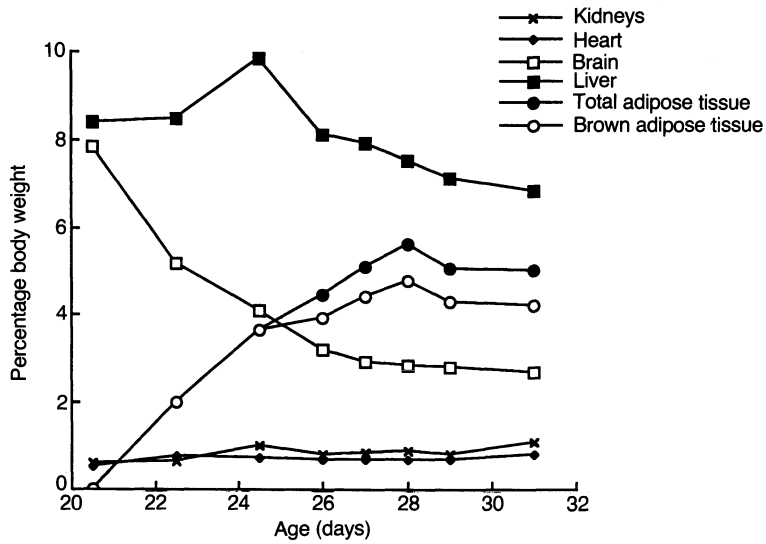

Figure 8.1 Organ weight as a percentage of body weight in the last third of gestation in rabbits.

imply therefore that it is in some way inadequate would be misleading.

8.1.3 THE RATE OF INTRAUTERINE GROWTH

Table 8.3 gives the length of gestation, birth weight and mean rate of growth of a range of mammals (Widdowson, 1970).

Whilst the larger mammals, weight for weight, do not achieve fetal development at the rate of the smaller mammals, their growth rates, in terms of weight gain per day, are remarkable. The fetal calf grows at 10 times the rate of the human fetus. Also shown in Table 8.3 are the rates of growth after birth whilst the young mammal is suckling. Again, the relatively slow-growing human young stands out as the exception.

Figures 8.1 and 8.2 give the organ weights in the last third of gestation in rabbits and man.

Table 8.4 gives the organ sizes of the mammalian fetus at birth.

Similar data are available for carcass and organ compositions. The human newborn, with its relatively large brain and high lipid content (Widdowson, 1950), is the exception, but then the human adult has a relatively large brain as well (Cross, 1979). The need to supply the requirements of the growing fetal brain has implications for transplacental transfer.

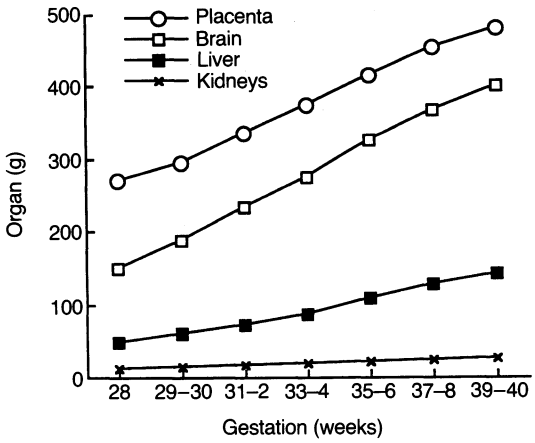

Figure 8.2 Fetal organ weight in man in the last third of gestation.

There are wide variations with respect to the amount of stored energy at birth. Most fetal mammals contain generous stores of glycogen in the liver and in muscle, but the amount of energy stored as fat varies widely (Shelley, 1961). By making assumptions about the metabolic rate, crude calculations can be made of starvation survival times after birth. Here again the human infant, with its relatively large store of triacylglycerol in white adipose tissue, is the exception (D. Hull, 1976) (Table 8.5).

Most other mammals are very dependent on the early establishment of oral feeding with the ready absorption of nutrients from the bowel.

This type of information is essential to the understanding of fetal growth and to any attempts to improve fetal development by modification of the maternal diet. These examples illustrate the wide variations between species and underline the differences in placental performance. They also illustrate why certain organs and systems are more vulnerable at various stages in development. The timing of these 'critical periods' differs between species with respect to phase in gestation and whether or not they occur before or after birth (Barker *et al.*, 1990; Lucas, 1991; Prentice, 1991; Atkinson, 1992; Steer *et al.*, 1992). The organ of major concern in this regard is the brain (Figure 8.3) (Dobbing and Sands, 1979).

The weight of the fetus at term, like the weight of the mother, is subject to biological variation (Figure 8.4).

In mammals, which usually produce a number of young, the fetal weight is increased if the litter size is very small and it may be reduced if it is very large. The values for rabbits are shown in Figure 8.5.

This reinforces the view that the environment provided by the mother determines fetal growth, and interest has been directed to, amongst other things, maternal nutrition (De Prins *et al.*, 1988; Prentice, 1991; Stephenson *et al.*, 1993a), uterine blood flow (F.C. Battaglia and Meschia, 1986), placental size (Mellor, 1984), placental position (Stevens, 1975), placental perfusion and umbilical blood flow (Stephenson *et al.*, 1990a). The studies are extensive, and there is no doubt that the severe manipulation of any of these factors will limit fetal growth. Which factors actually operate in small or large litters, or contribute to the production of a runt, or to intrauterine growth retardation in the human infant, are still not resolved. Nor can it be doubted that there are many factors within the fetus which determine and control its growth rate both in normal and disordered states (Van Assche and Robertson, 1982; Gluckman and Liggins, 1984).

8.1.4 THE CHALLENGE OF BIRTH

To complete this introduction to fetal physiology, it has to be recognized that the fetus not only establishes a pattern of growth to enable it to achieve adult size and performance, and maintains and controls its bodily systems within the intrauterine environment, but it also prepares itself for the challenge of the birth experience and the adjustment and acceleration of some functions after birth. Thus, an individual organ or structure in-

Table 8.4 Organ weights in newborn animals as a percentage of birth weight.

	Liver	*Muscle*	*Skeleton*	*Skin*	*Heart and lungs*	*Brain*
Sheep	2.1	22	23	11	2.8	1.3
Man	**4.0**	**18**	**14**	–	**2.3**	**10–13**
Pig	4.7	30	24	10	2.2	3.7
Rabbit	6.6	15	–	19	3.4	2.9
Rat	6.3	15	–	15	–	5
Mouse	4.3	–	–	–	1.9	6.3

Table 8.5 Theoretical survival times based on the amount of stored energy at birth assuming the newborn stayed in a thermoneutral environment. (From Hull, 1976.)

	Lipid stores (g/kg body wt)	*Carbohydrate in liver and muscle (g/kg body wt)*	*Oxygen consumption (ml O_2/kg/min)*		*Theoretical survival times (days)*
			Minimum	*Maximum*	
Lamb	30	11	10	30	6
Man	**160**	**11**	**6**	**16**	**35**
Piglet	11	23	12	36	2.2
Rabbit	58	5	220	60	4
Rat	11	8	25	50	0.8

creases in size, develops, changes and modifies its functions and prepares itself for a major change in environment. Enzyme systems are induced in anticipation of birth; if they are not induced the system they support may fail. A prematurely delivered mammal may die not because a system, say the lung, is not anatomically developed, but because critical enzyme systems have not been induced, for example to enable the secretion of surfactant (Avery and Mead, 1959). For information on this and other aspects of the biochemical development of the fetus, the reader is referred to C.T. Jones (1982).

8.1.5 METHODOLOGIES

Our knowledge of fetal physiology is derived from a variety of sources and in this subject, perhaps more than in others, the original observations do not always justify the constructions that are put upon them. The problem is that the fetus is difficult to reach without interfering with it in some way. Much valuable information has been obtained by structural analysis of fetuses delivered normally or by Caesarean section of mothers kept under different conditions. Many observations have been made after experimental manipulation of the placenta in some way, for example tying a uterine artery in rats (Wigglesworth, 1964), or removing cotyledons in sheep (Mellor, 1984); and after experimental procedures on the fetus, for example removing the thyroid or occluding the trachea and allowing the pregnancy to continue (Jost, 1954). However, these studies of classical physiological mechanisms were made on the exteriorized fetus from an anaesthetized mother and most often the sheep has been the species employed (Dawes, 1968). The placental performance under such conditions was unlikely to be ideal, which led to the development of techniques to study the unanaesthetized fetus in the unanaesthetized mother by inserting catheters into various fetal channels, particularly the umbilical vessels, and allowing both mother and fetus

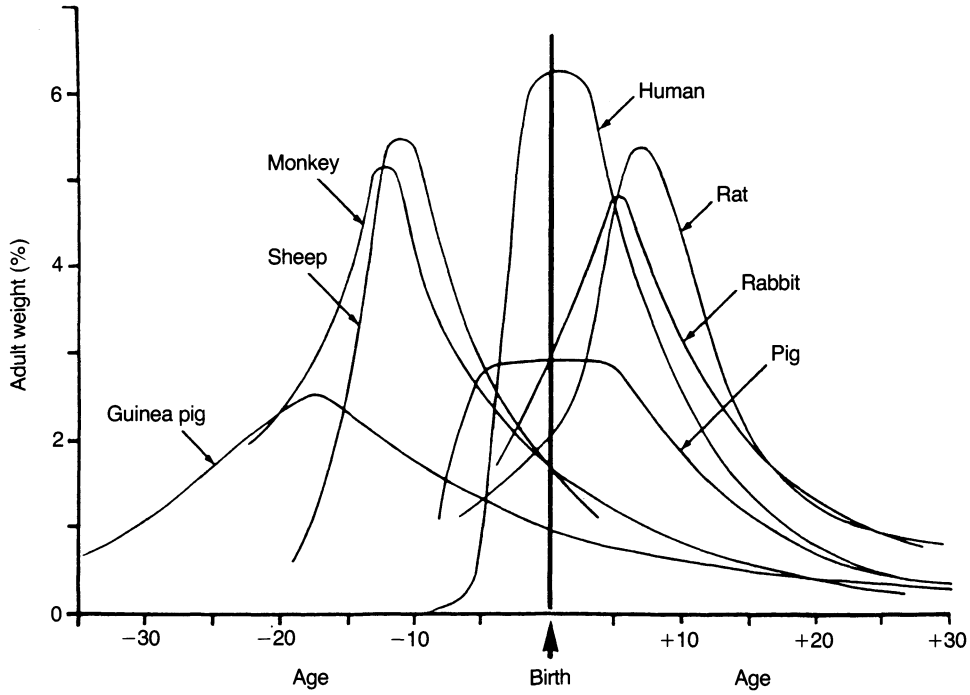

Figure 8.3 The brain growth spurts of seven mammalian species expressed as first-order velocity curves of the increase in weight with age. Rates are expressed as weight gain as a percentage of adult weight for each unit of time. The units of time for each species are as follows: guinea pig, days; rhesus monkeys, 4 days; sheep, 5 days; pig, weeks; man, months; rabbit, 2 days; rat, days.

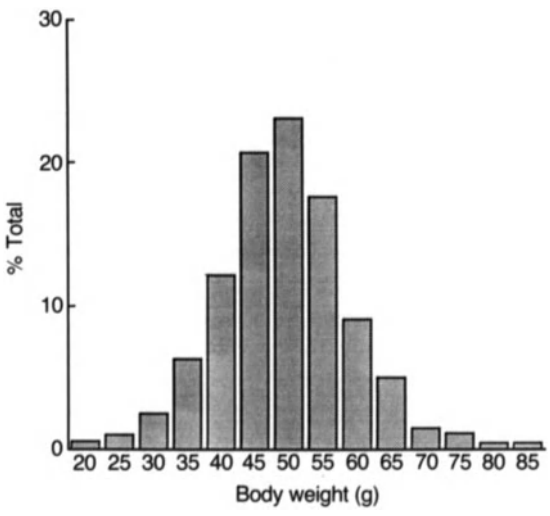

Figure 8.4 Distribution of birth weights of newborn rabbits.

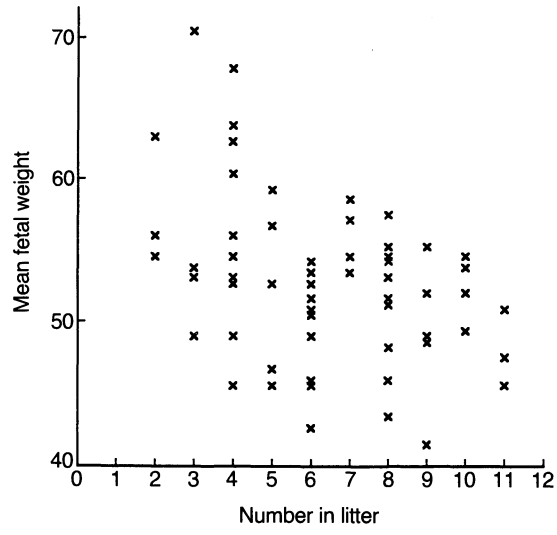

Figure 8.5 Mean fetal weight of rabbits in litters of different sizes.

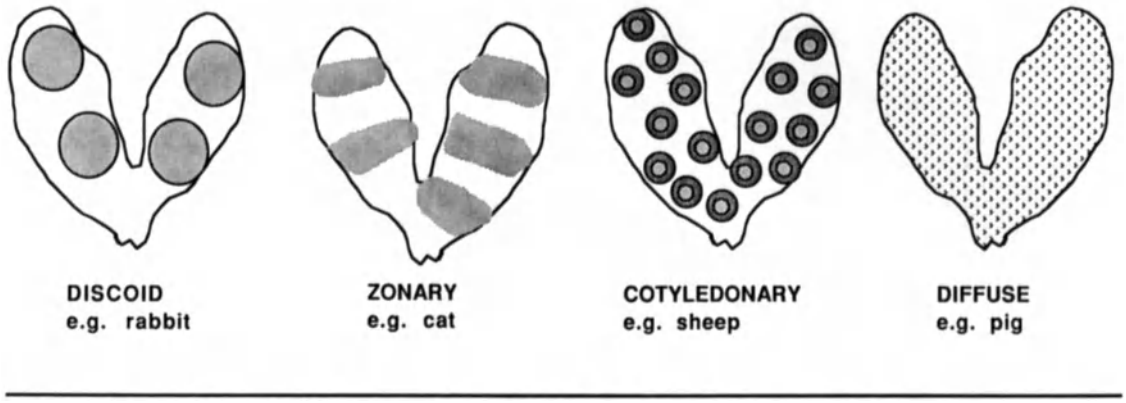

Figure 8.6 The different macroscopic structures of the mammalian placentae.

to recover (Rudolph and Heymann, 1980). This technique has been most successful in the fetal sheep, and much of our precise understanding of fetal physiology relates to that of the lamb (F.C. Battaglia and Meschia, 1986). A great deal of the work relating to placental transfer has been undertaken in the sheep, a mammal which is attractive experimentally because its large size allows repeated blood sampling and because the relatively unresponsive ovine uterus is invaluable in establishing chronically catheterized preparations. It must be remembered that the lamb (and in addition the sheep epitheliochorial placenta) has its own characteristics and, despite being of similar size to the human fetus, differs in a number of important ways.

Interpretations have also been made on spot samples taken from the fetus, for example during clinical procedures, and from studies on cord blood samples collected at birth. These have to be interpreted with caution but are sometimes all that is available. Measurements using isotopes in animals have given valuable information, but because of the many fluxes and spaces involved, calculations are not easy (Nathanielsz, 1980). The pre-term infant is mentioned briefly in section 8.4.5. However, whilst the prematurely born infant, after a brief period of adjustment, follows a similar growth curve as the fetus, its physiology is very different and provides very little information to help us understand intrauterine life.

8.2 THE PLACENTA

8.2.1 STRUCTURE

The **naked eye** appearance of mammalian placentae differs strikingly (Figure 8.6) (Stevens, 1975).

Organs such as the kidneys, liver, lung and brain are readily recognized, irrespective of the species; not so for placentae. The human placenta is shaped as a single thick dish (the word placenta means plate) which attaches itself to part of the uterine wall. Usually, the position is favourable, but occasionally it is not. It may settle over the cervical os and therefore bleed as labour commences and obstruct the outlet. Some monkeys have two such discs, whereas the sheep has many suction pads attached to predetermined sites (caruncles) whose supply and draining vessels join to form the umbilical vein and arteries. The dog and cat have placentae shaped like a collar which ring each fetus as it lies in

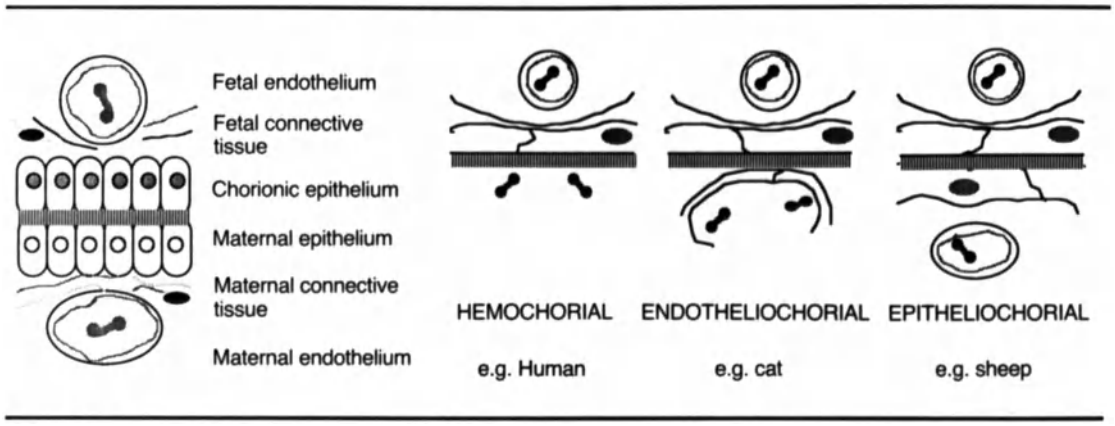

Figure 8.7 The microscopic structure of mammalian placentae.

the uterus, whereas the pig and the horse have a diffuse placenta that spreads all over the inner surface of the uterus. The significance or consequences of these anatomical variations is not known. For more on the subject of placentation see Chapter 4 of this volume.

There are equally striking differences in the **microscopic structure** (Figure 8.7).

The basic arrangement consists of a maternal blood vessel, connective tissue and epithelium, next to fetal epithelium, connective tissue and blood vessel. In some placentae all three are preserved, in others various maternal layers disappear; in man and rabbit, for example, no maternal layer of cells separates the maternal blood from fetal tissue so in that situation the whole of the placenta is a fetal organ under the direction of fetal genes. The placental barrier, that is the interface that chemicals have to cross to reach or leave the fetus, may therefore be anything from three to six cells thick. There does not seem to be any detectable difference in the transfer rates of oxygen or nutrients that obviously relate to structure; electrolytes and fatty acids may be different (Stephenson *et al.*, 1990b). The fetal–maternal interface is not of uniform thickness. Water and water-soluble substances cross the placental cells with more difficulty than fat and fat-soluble substances (Sibley and Boyd, 1988). Water is filtered through the cells, and between cells; the relative amounts of the two routes vary with the species (Stulc, 1988).

The capacity of the placenta as an organ of exchange is clearly going to be dependent on the **blood supply** to the two sides and their interface with each other. Here again there appear to be differences between species. Many have attempted, by anatomical studies, to disentangle the vascular arrangements to determine whether the direction of maternal and fetal blood flows run together, i.e. concurrent flow, or against each other, countercurrent. For an explanation of the implications of these arrangements and how the countercurrent arrangement works to maternal or fetal advantage depending on the relative blood flows the reader is referred to F.C. Battaglia and Meschia (1986).

The maternal blood supply transverses the myometrium in spiral arteries. As the human placenta implants these vessels are converted by intravascular cytotrophoblastic cells into wider uteroplacental vessels, which gently squirt maternal blood into the intervillus space. The vascular villus tissue begins to form by 3 weeks post conception. It then undergoes growth and arborization until it reaches its definitive shape by 16 weeks' gestation, after which time it grows in size and

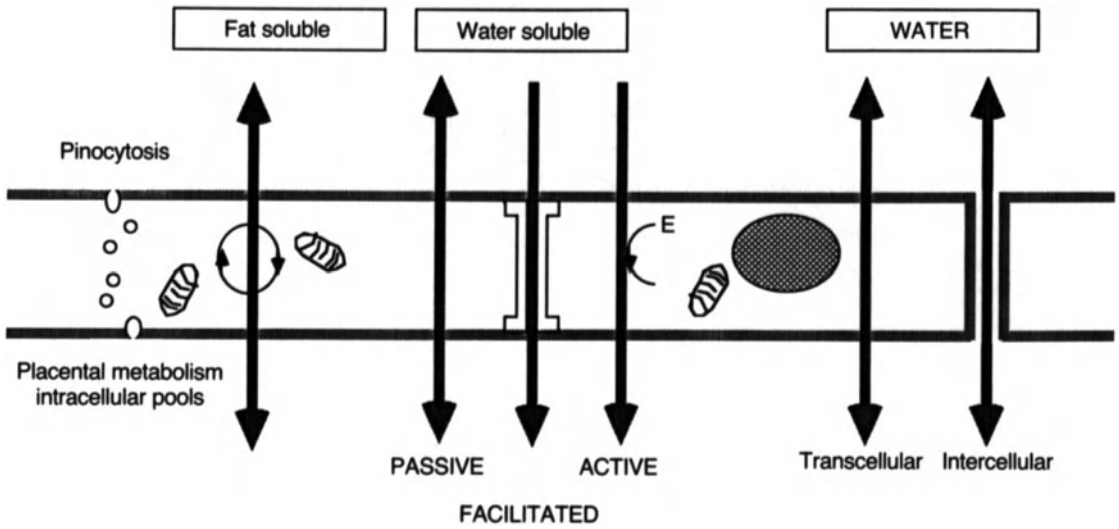

Figure 8.8 Modes of placental transfer.

complexity until term. One imagines the villus fronds washing about in maternal blood like seaweed in a troubled sea. This arrangement is closest to concurrent flow (Faber and Thornburg, 1983) or 'venous equilibration', and in that it resembles the sheep, which by contrast has a maternal epithelial layer. The horse has a placental countercurrent arrangement that works to 'fetal advantage', and the guinea pig one that works to 'maternal advantage'.

8.2.2 TRANSFER

The transfer of a substance from mother to fetus or vice versa may depend on, amongst other things:

1. The concentration of the substance in the delivery component of the maternal circulation; for glucose it is the level in the serum, for oxygen the partial pressure (Faber and Hart, 1966), and for fatty acids it is probably the concentration in the free fatty acid compartment.
2. The mechanism of transfer across the placental cell membranes, for example receptor sites (Sibley and Boyd, 1988).
3. The concentration in placental intracellular pool or pools.
4. The availability of appropriate carrier proteins if required (Stephenson *et al.*, 1993a).
5. The placental consumption of the substance.
6. The concentration in the blood feeding the fetal side of the placenta (Stephenson *et al.*, 1990b).

Substances cross by different routes; the transfer may be passive, facilitated or energy dependent or occur by pinocytosis (Figure 8.8).

(a) Water

Water diffuses across the placenta, either traversing the cells (transcellular) or running between the cells (paracellular). Some indication of the size of the paracellular route can be gauged by the diffusion coefficients of marker molecules. This has led to the view that the pores must be larger in the human haemochorial placenta than in the sheep epitheliochorial placenta (Boyd *et al.*, 1976; Faber and Thornburg, 1983; Stulc, 1988). Electrolytes in high concentrations in serum

such as sodium and chloride, will pass easily down the paracellular channels and equilibrate between the two sides.

Using labelling techniques, Hutchinson *et al.* (1959) calculated that 3.6 l/h was the transfer rate in the term human fetus weighing around 3.2 kg, and that 150 ml/h was transferred between the fetus and the amniotic cavity. The term fetus contains around 2.5 l, and accumulates water at a rate of 20 ml/day. Thus, the net transfer to the fetus is a very small fraction of the diffusional exchange (Hytten and Leitch, 1971; Hytten, 1979).

In the unstressed state, the total osmotic pressure in the fetal blood is below that in the mother, and it is not clear what counterbalances this. The pressure within the perfusing vessels may be a factor, as may the flow characteristics across the pores (see F.C. Battaglia and Meschia, 1986). If maternal osmotic pressure is lowered by infusion of hypotonic solutions, the fetus gains water (Tarnow-Mordi *et al.*, 1981); if hypertonic solutions are infused, then the fetus shrivels a little.

(b) Electrolytes and minerals

With the haemochorial placenta, the main extracellular electrolytes sodium and chloride cross to the fetus easily (Canning and Boyd, 1984). As with water, the bidirectional fluxes are much greater than the net flux; the main exchange is probably via the paracellular route, by which route water and electrolytes presumably move together as they do in serum. Water filtering across the cells is thought to be solute free, and sodium and chloride pass through in relatively small amounts. In the epitheliochorial placenta of sheep, the diffusion permeability is very low and may be controlled and in turn determine fetal growth (Faber and Thornburg, 1983). In the epitheliochorial placenta of the pig, sodium flux is thought to be unidirectional and energy dependent (Sibley *et al.*, 1986). Potassium transport in guinea pigs appears to be energy dependent (D.J. Bailey *et al.*, 1979).

(c) Calcium and phosphate

The growing fetus needs calcium to form bone. In the fetal sheep, the accretion rate has been calculated to be around 200 mg of calcium per kg per day. It also seems that in the fetal sheep there is mainly a one-way flow of calcium to the fetus, but in the monkey only around 20% of the calcium which enters the fetus is retained. Despite this net flux to the fetus in all mammals studied, the maternal concentrations of total plasma calcium and ionized calcium have been **below** fetal levels. Therefore, calcium does not flow down a pressure gradient to be taken up by fetal bone as required. Both calcium and phosphate are transported by carrier-mediated energy-dependent mechanisms (Care and Ross, 1984; Lajeunesse and Brunette, 1988; Stulc, 1988). There is some evidence that this active process is under the control of the vitamin 1,25-dehydroxycholecalciferol. This seems to be made in the maternal kidney and to be transferred across the placenta (Clements, 1988), and both the placenta and the fetal kidney have the capacity to convert it into its active form. By contrast, calcitonin and parathyroid hormone do not cross the placenta. As with many fetal systems they develop along with the structures they control. The parathyroid gland becomes active in early fetal life and is presumably involved in bone resorption and calcium homeostasis from an early stage (Brunette, 1989).

(d) Iron

Maternal serum transferrin, the carrier protein for iron, is the main source of fetal iron. The transferrin probably enters the placental cells by endocytosis, which is receptor mediated. The iron presumably enters an intra-

Table 8.6 Partial pressures, saturation and content of oxygen in the blood taken from the maternal uterine artery and vein and fetal umbilical artery and vein.

	PO_2 *(mmHg)*	*Oxygen saturation (%)*	*Oxygen content (vol. %)*
Maternal uterine artery	90	95	14.5
Maternal uterine vein	35	70	10.5
Fetal umbilical vein	28	65	13.0
Fetal umbilical artery	15	25	5.0

cellular pool before being released into the fetal circulation (Van Dijk, 1988).

(e) Blood gases

Table 8.6 shows the pressure gradient of oxygen as it travels from mother's blood to fetal blood.

The partial pressure of oxygen in fetal blood leaving the placenta (oxygenated but travelling in a vein) is relatively low compared with that leaving the lungs of a newborn infant (Comline and Silver, 1975). Nevertheless, because of the characteristics of the fetal haemoglobin–oxygen dissociation curve (section 8.3.2) and the relative polycythaemia of the fetus, the oxygen content is still high. Table 8.6 also gives the values for oxygen saturation and content of blood in the maternal and fetal circulations. The difference in oxygen dissociation combined with the difference in haemoglobin concentrations (the fetal haemoglobin concentration being higher than the mother's) results in the oxygen content in maternal uterine vein blood being lower than that in umbilical vein blood.

The rabbit placenta has a countercurrent system and is the most effective so far studied with respect to gas exchange (Faber and Hart, 1966). The primate placenta probably has a countercurrent system also, as have the guinea pig and the mare. The fact that umbilical venous oxygen tension can exceed that in the uterine vein is evidence in favour of this. Although the sheep cotyledon was originally thought to conform to the countercurrent pattern, more recent evidence has suggested that it may function as a double-pool venous equilibrator, a cross-current arrangement, or a mixture of concurrent and countercurrent exchanger types (Metcalfe *et al.*, 1965; Faber and Thornburg, 1983; F.C. Battaglia and Meschia, 1986). It also seems unlikely that the cow, another ruminant, possesses the countercurrent exchanger.

Maternal arterial blood gases do not vary greatly between species, but the blood gas tensions in the fetus do vary. The different uterine vein–umbilical vein PO_2 gradients in different species may reflect the range of exchanger types in the mammalian kingdom (Meschia *et al.*, 1967; Metcalfe *et al.*, 1967). This is an easier subject to speculate on theoretically than to elucidate experimentally, and much work remains to be done. Nevertheless, it now seems clear that the countercurrent arrangements found in man and rabbit can be exploited preferentially by either mother or fetus. This was predicted mathematically, and it has been demonstrated that the guinea pig countercurrent placenta works to 'maternal advantage' (a lower maternal placental blood flow for a given rate of placenta transfer compared with a concurrent arrangement), whereas the countercurrent placenta of the mare operates to confer 'fetal advantage' (a higher fetal substrate concentration for a given flow rate).

The fetus does not store oxygen. Therefore, it is dependent on a continuous supply and it must have a robust and efficient delivery system to survive. Considering its complexity, it is remarkably efficient. There is, however, an element of arteriovenous shunting in both maternal and fetal circulations, analogous perhaps in physiological terms to

Table 8.7 Oxygen consumption of the placenta and of the fetus in sheep. (From Battaglia and Meschia, 1986.)

	Oxygen content (μmol/ml)		
Maternal uterine artery	6.00		
	−	Uterine flow	1.35 × 1600
Maternal uterine vein	4.65	(1600 ml/min) =	2160 μmol/ml (maternal to placenta)
Fetal umbilical vein	5.45		
	−	Umbilical flow	1.47 × 800
Fetal umbilical artery	3.98	(800 ml/min) =	1176 μmol/ml (placenta to fetus)

the ventilation–perfusion mismatch which occurs in the lungs. The placenta itself has a high demand for energy; the figures calculated by F.C. Battaglia and Meschia (1986) for the sheep placenta are shown in Table 8.7.

This appears to be what economists call 'top slicing', but it is not clear that the placenta inevitably has priority.

The transfer of oxygen does not appear to be limited by the diffusion characteristics of the barrier. Rather, the restraints rest with supply and uptake. The supply taps directly into the maternal arterial supply, but it can be reduced by factors which interfere with uterine perfusion, and these include any stimulus which provokes sympathetic nervous activity or the release of catecholamines. Apparently uterine blood flow is optimal in the resting state.

The umbilical circulation is unique in that it appears to have no innervation and the umbilical artery shows little or no response to changes in partial pressures of oxygen, carbon dioxide, pH or catecholamines. Hormones such as angiotensin II, vasopressin or prostaglandins may play a physiological role in placental fetal vascular resistance (e.g. Tuvemo, 1980). However, anyone who has attempted to catheterize the umbilical artery will have no doubts about its ability to contract. Notwithstanding this, the current position appears to be that the resistance in the major vascular bed of the fetal circulation, namely that through the placenta, is determined primarily by its anatomy.

Thus, given a fixed umbilical flow, a flow-limited transfer across the placental membranes and a supply pool formed from maternal arterial blood, the oxygen content of the umbilical vein will remain fairly constant, and the oxygen content in the umbilical artery will directly and immediately reflect fetal oxygen consumption. The way the fetus appears to exert control over its oxygen supply is by changes in the umbilical arterial partial pressure.

The robustness of the system is reflected by its reserve capacity. This is illustrated by the clinical condition of rhesus incompatibility, in which maternal antibodies cross the placenta and destroy fetal red cells, causing the fetus to become anaemic. The fetal haemoglobin can fall to one-third of normal and the fetus still survives.

Carbon dioxide also diffuses readily across the placental membranes, and the diffusion capacity is some 20 times that of oxygen. The umbilical artery/maternal artery difference is around 5 mmHg. In women, this pressure difference is in part due to the lowering of the

Table 8.8 Glucose concentration in maternal and fetal blood. (From Shelley, 1979.)

	Blood glucose (mg/dl)			
	Maternal	*Fetal*	*Ratio*	*Source of fetal blood*
Sheep	60	15	4.0	Umbilical catheter
Cow	70	23	3.0	Umbilical catheter
Horse	80	40	2.0	Umbilical catheter
Rabbit	58	40	1.5	Neck vessels
Man	**71**	**63**	**1.3**	**Scalp stab**

maternal PCO_2 due to increased pulmonary ventilation, which gradually increases towards term.

(f) Glucose

Glucose is subject to fairly strict homeostatic control in the maternal circulation and thus it is always present on the maternal supply side. Glucose is as constantly and readily available as oxygen. On average, the fetal serum concentration of glucose is lower than that in the maternal circulation but the average concentration gradient varies widely between species (see Table 8.8; Shelley, 1979).

Glucose passes through the placenta as it does into red cells, that is at a faster rate than might be expected on the basis of simple diffusion alone and certainly faster than many chemicals of similar structure and size. There are 'doors' that open only to glucose because there is a carrier system which binds glucose molecules selectively. The evidence that glucose crosses the placenta by facilitated transfer is compelling (Shelley, 1979). It is the nature of facilitated transfer that it has a limit, that it can be saturated and that it can also be reduced by competition with like chemicals. Given those characteristics, transfer will be determined by the maternal–fetal gradient, the blood flow on both sides and by the morphology of the placenta. But is it subject to further control? Fetal pancreatectomy led to intrauterine growth retardation (Fowden *et al.*, 1984; Fowden, 1989), although there was relative sparing of brain growth, and decreased fetal uptake of the glucose from the placenta. However, it was unclear whether this decreased uptake was simply due to a decreased gradient across the placenta, because of the fetal hyperglycaemia following pancreatectomy, or was mediated via decreased stimulation of the insulin receptors on the placenta (Posner, 1974; Witsett *et al.*, 1979; Potau *et al.*, 1981). It is not known whether these insulin receptors are on the fetal or maternal surface of the placenta. In the experiments by Fowden and co-workers (Fowden, 1989), fetal glucose utilization fell by half, presumably because of the impaired entry of glucose into cells in the absence of insulin. However, approximately half of fetal glucose metabolism appeared not to be insulin dependent, although this proportion might have been raised artefactually by the fetal hyperglycaemia (see above) and hence an increased gradient for transfer of glucose into cells.

In contrast, when fetal insulin concentrations were increased (Kervan and Girard, 1974), umbilical glucose uptake was enhanced (F. Battaglia and Hay, 1984). This occurred whether or not the fetal hyperinsulinaemia was created by an exogenous fetal insulin infusion (Bassett and Madill, 1974; Simmons *et al.*, 1978) or by the stimulation of endogenous fetal secretion (R.D.G. Milner and Hales, 1965; Kervan and Girard, 1974; Phillips *et al.*, 1981). The placenta possesses both insulin (Posner, 1974) and somatomedin receptors, which may promote placental growth (Demers *et al.*, 1972) or the transfer of

nutrients across the placenta (Gluckman and Liggins, 1984). Whilst many other hormones have been shown to exert an effect on fetal metabolism and substrate utilization (Fowden, 1980; Fowden *et al.*, 1990), few studies have investigated their effects on placental transfer.

As with oxygen, the placenta takes what glucose it needs before passing on the remainder (F.C. Battaglia and Meschia, 1988). In the lamb, the placenta consumes glucose at twice the rate of the fetal body and double-labelling experiments suggest that much of the glucose (and some of the amino acids) for placental metabolism is taken up from the fetal circulation and not directly from the maternal circulation (F.C. Battaglia and Meschia, 1986). The lamb placenta makes lactate and releases it into both the maternal and the fetal circulation, which makes a significant contribution to the energy supply to the fetus, around 25%. Lactate may be an important source of energy in other mammals and its presence in the fetal circulation does not necessarily indicate anaerobic metabolism.

(g) Fructose

Fructose, unlike glucose, does not cross the placenta, which emphasizes that the facilitated transport of glucose is specific and the carrier will not bind any hexose molecule. Some fetal animals make fructose but do not readily use it as an energy source, so-called 'fructogenic species' (e.g. the lamb). Fructose is found in the fetal circulation of those species which have placentae which are relatively impermeable to sodium, potassium and chloride (e.g. the sheep and goat). The supplies of these electrolytes are only ensured by steep concentration gradients across the placenta. Fructose increases the osmotic pressure in the fetal circulation, and it is postulated that this may act to offset the osmotic pressure generated by the higher electrolyte concentrations on the maternal side of the placenta (Faber and Thornburg, 1983; Canning and Boyd, 1984). Fructose is not present in significant amounts in the fetal plasma of species with a haemochorial placenta in which electrolyte diffusion permeabilities are much higher, and in 'fructogenic species' plasma fructose concentration falls rapidly after birth (F. Battaglia and Hay, 1984). Both of these observations are consistent with the proposed role of fructose in transplacental osmotic balance. The only other suggestion for a potential role for fructose has come from studies showing that fetal fructose concentrations fall as maternal fasting progresses in pregnant sheep. Perhaps fructose represents a slowly mobilized substrate for gluconeogenesis by the fetus (Hay, 1979).

(h) Amino acids

This mixed group of organic compounds, characterized by the presence of nitrogen, are the building blocks of protein; the fetal proteins are formed from amino acids which cross the placenta. Amino acids travel free in the circulation. The concentrations in the maternal circulation are lower than those in the fetal blood, but both are lower than the placental intracellular concentrations, which inter-relate with protein turnover in trophoblast cells. Table 8.9 gives the main amino acids and indicates the mechanisms of placental transfer, which differ depending upon the form of the amino acid, some of which are actively transferred.

Placental and fetal tissue metabolize and change amino acids depending in part on protein turnover. Protein turnover in the placenta and fetal tissues occurs at 10–20 times the rate in maternal tissues.

The net flux of most, but not all, amino acids is from the placenta into the fetal circulation. The data for sheep reported by Lemons *et al.* (1976) show that the net flux does not mirror the fetal requirement for structure. For example, glutamine passes from the fetal circulation **into** the placenta.

Table 8.9 Transport of amino acids across the placenta.

	Metabolism	*Examples*	*Transport*
Acidic	Purine and pyrimidine synthesis	Aspartate Glutamate	Little transported
Neutral	Glucogenic	Glycine Alanine	Active Energy dependent
	Ketogenic	Valine Leucine	? Passive
Basic	Protein structure	Lysine Histidine	Active Energy dependent

Table 8.10 Maternal and fetal plasma fatty acids (mmol/l).

	Maternal plasma	*Fetal/Newborn plasma*		*V–A difference*
		Umbilical vein	*Umbilical artery*	
Pig	0.217	0.037	0.024	±
Cat	0.320	0.190	0.180	±
Sheep	0.578	0.097	0.124	±
Man	**1.010**	**0.242**	**0.206**	+
Rabbit	1.09	1.10	0.70	+

The acidic amino acids do not appear to cross the placenta in significant amounts, and aspartate and glutamate are presumably made from asparagine and glutamine. The basic amino acids appear to be transferred in amounts just sufficient for structure. The neutral amino acids, in particular, are transferred into the fetal circulation in excess of fetal requirement and are thought to be an energy source (M. Young, 1979; F.C. Battaglia and Meschia, 1986).

(i) Fatty acids

Glucose is the main source for fetal cellular energy in man, and a major source in lambs, and amino acids are essential for the construction of protein, so it is not unexpected that both have been shown to cross the placenta in substantial amounts in all species studied, and it would be most unlikely that the same is not true for all mammals. The position with respect to fatty acids is different. In some species, the placenta is relatively permeable to fatty acids, in others it is not.

Table 8.10 gives the free fatty acid concentrations in maternal and fetal circulations of a number of species.

It does appear that those placentae with maternal layers, e.g. lamb, pig and cat (Elphick *et al.*, 1979, 1980; Elphick and Hull, 1984), are not permeable but that those without are permeable. However, the horse, with a six-layer placenta, is an exception to this generalization (Stammers *et al.*, 1987). In those species which let fatty acids through, the transfer appears to be related to maternal free fatty acid concentrations, and if the maternal level goes up, as it does with stress, starvation and labour, then more fatty acid

flows to the fetus (Hershfield and Nemeth, 1968; Elphick *et al.*, 1978a,b; Haugel *et al.*, 1988; Stephenson *et al.*, 1990b). Some mammals voluntarily starve themselves prior to delivery, the rabbit being an example; the effect is to fatten the fetus and thus increase the energy stores of the newborn at birth (Edson *et al.*, 1975). Fatty acids in exogenous and endogenous triacylglycerol fractions may also be available to the placenta and so to the fetus (Stephenson *et al.*, 1993b). Certainly the maternal free fatty acid fraction does not appear to be the sole source in rabbits (Stephenson *et al.*, 1990b).

In the main, fatty acids in the free fatty and triacylglycerol serum fractions reflect dietary lipids, which are of variable chain length, mainly between 14 and 20 carbon atoms, with either zero, one, two or three unsaturated bonds. Linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) are essential fatty acids and important for the structure of membranes in all tissues, including the brain (Anderson *et al.*, 1974; Brenner, 1984; Carlson *et al.*, 1986; Brody *et al.*, 1987; Koletzko *et al.*, 1989; Uauy *et al.*, 1990). In general, the differing fatty acids cross the placenta in proportion to their concentrations in the maternal free fatty acid compartment, but there are exceptions, and the flux may depend on competition for carrier proteins (Brandes *et al.*, 1987; Stephenson *et al.*, 1993a). Arachidonic acid ($C_{20:4}$), the precursor of prostaglandins, may be made by desaturation of linoleic acids in the placenta or by selective uptake of arachidonic acid from maternal phospholipids, as arachidonic acid enters the fetal circulation in excess of that which might be expected from maternal concentrations. The problem remains as to how the essential fatty acids reach the fetal sheep or fetal pig, for significant arteriovenous differences in free fatty acids have not been detected.

With the exception of arachidonic acid and, in one uncorroborated study, oleic acid (Hendrickse *et al.*, 1985), the evidence points to non-selective transfer of free fatty acids across the human and rabbit placenta. Non-selective uptake by the placenta from the maternal circulation does not easily explain the high percentage of arachidonic acid found in the free fatty acid fraction of the placenta itself (Robertson and Sprecher, 1968). The free fatty acid composition of the placenta is sufficiently different from that of the maternal plasma to suggest that at least some components of the placental free fatty acid pool are derived from other sources. It seems more likely therefore that the placenta alters the pattern of free fatty acids presented to it before they enter the fetal circulation rather than there being a selective mechanism favouring transplacental transfer of particular longer chain fatty acids. The human placenta has the capacity to elongate and desaturate $C_{18:2n6}$ (linoleic acid) to $C_{20:4n6}$ (arachidonic acid) *in vitro* (Zimmerman *et al.*, 1979). Alternatively, maternal phospholipids are rich in 20:4n6 and human and rat placentae have been shown to contain the phospholipases necessary for release of polyunsaturated fatty acids from phospholipids (Robertson and Sprecher, 1967; East *et al.*, 1975). In infants with severe intrauterine growth retardation, or those born to women with pre-eclampsia, Mead acid has been consistently found in the fetal plasma. This is considered to be a marker for severe malnutrition in adults (Rivers and Frankel, 1981; Orchard *et al.*, 1983).

Very different results were obtained in the sheep (Leat, 1966). The profile of fetal plasma lipids suggested synthesis from non-lipid precursors and the degree of unsaturation of free fatty acids was consistent with a relative paucity of essential fatty acid transfer across the placenta from the ewe. Although the sheep placenta has a very low permeability for fatty acids, there is a suggestion that even in this species essential fatty acids are handled differently from non-essential fatty acids. In experiments on chronically catheterized fetuses in which [^{14}C] linoleic acid and [^{3}H] palmitic acid were infused intravenously

into three ewes, only traces of label crossed the placenta, but these traces were almost entirely confined to ^{14}C (Elphick *et al.*, 1979). Although the data suggested that the ^{14}C label had crossed the placenta as [^{14}C] formate and [^{14}C] acetate (i.e. short-chain metabolic breakdown products of linoleic acid rather than intact linoleic acid), virtually no ^{3}H-labelled products derived from the non-essential palmitate appeared in the fetus. The linoleic acid content of fetal lamb adipose tissue, liver, lung and cerebellum was very low (Elphick *et al.*, 1979), and these authors estimated that, although only a maximum of 4 mg/day of linoleic acid could be transferred across the sheep placenta, this might be sufficient to account for the 500 mg of linoleic acid in a term lamb.

Amounts of other longer chain free fatty acids ($C_{22:5n3}$ and $C_{22:6n3}$ in addition to $C_{20:4n6}$) over and above the amount predicted from non-selective transfer have been found in the pig (Elphick *et al.*, 1980), a species in which transfer of free fatty acids is generally very low. Examination of the published data from the sheep (Elphick *et al.*, 1979) shows that, although there was no evidence of net transfer of free fatty acids into the umbilical circulation, $C_{20:4n6}$ accounted for a greater proportion of the free fatty acid fraction in fetal blood than maternal blood. It could be argued that in these species with multi-layered placentae relatively impermeable to lipids, there would have been a strong evolutionary advantage if essential fatty acids were handled more favourably. Presumably, this is because the fetus still requires essential fatty acids and these must be donated by the placenta even if the placental characteristics are otherwise hostile to lipid transfer.

In conclusion, the fetus draws from the placenta a variable mixture of glucose and amino acids for energy (with lactate in sheep) and amino acids and fatty acids for structure. We are only just beginning to understand the mechanisms involved and know little of the controlling factors. This section, at best, is a brief overview of a fascinating and complex biological system.

8.3 FETAL PHYSIOLOGY

8.3.1 HEART AND CIRCULATION

A circulation is essential for any complex organism; the developing fetus needs to draw nourishment either from the yolk sac or later from the maternal bloodstream via the placenta. In man, the heart tube begins to contract by the end of the third week of gestation and to achieve unidirectional flow by the end of the fourth. The beating heart can be recognized with ultrasonography by the seventh post-menstrual week of human pregnancy. Whilst it is pumping, the internal septa develop and the tube gently contorts to the shape found in the second half of gestation and in adult life.

Despite its form, the fetal heart acts more like a single pump. Both ventricles discharge the majority of their output into the systemic circulation, and ventricular function is often considered in terms of the combined ventricular output. The combined ventricular output is approximately 400 ml/min/kg in the near term fetal sheep (Teitel, 1992), which is approximately the same as the output of each ventricle when they are pumping in series after birth. There is, therefore, a dramatic increase in ventricular output after birth. The right ventricle ejects about two-thirds of the combined ventricular output in the fetal lamb, but in the human fetus this fraction is somewhat less because of the greater brain blood flow, which is supplied by the left ventricle. However, Doppler ultrasound studies in the human fetus have confirmed that the right ventricle is still predominant, and this is consistent with the 'right dominant' voltages seen in the chest leads of the neonatal electrocardiogram (O'Callaghan and Stephenson, 1992). Although the ventricles function in parallel in prenatal life, the chambers perform similar functions to post-natal life. The

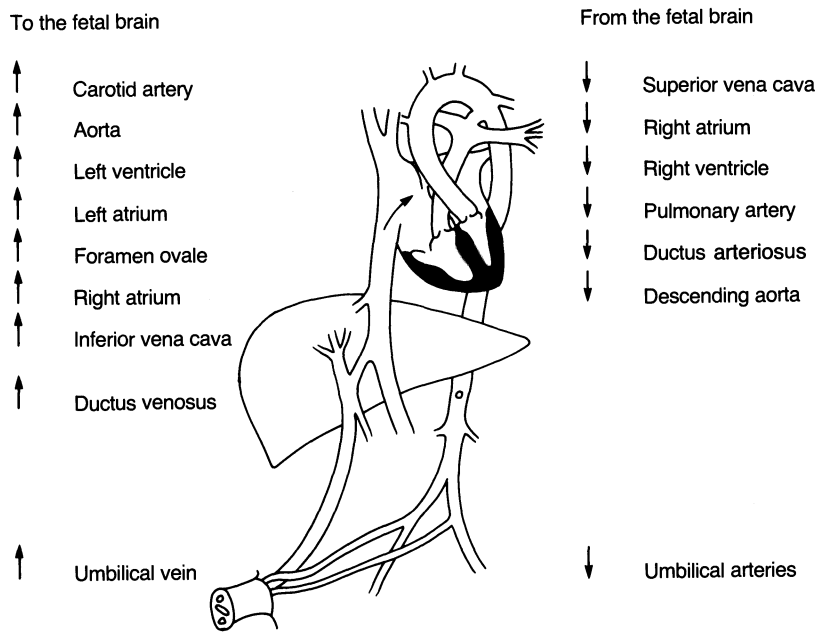

Figure 8.9 The fetal circulation.

fetal right ventricle primarily delivers blood for oxygen uptake (because post-ductal blood perfuses the placenta), just as in post-natal life the right ventricle pumps blood to the lungs; and the fetal left ventricle principally ejects blood for oxygen delivery (well-oxygenated blood, via the foramen ovale, for the coronary arteries and brain), just as the left ventricle supplies the systemic circulation after birth.

If one ventricle fails to form *in utero*, e.g. hypoplastic left heart, then the fetus experiences no difficulty but the condition is quickly fatal after birth. Very rarely in twins, the heart of one twin supports both of them so that one twin has no heart at all (acardia).

(a) Distribution

It is not so much the structure of the heart and vessels as the route of oxygen supply which is so different in the fetus (Figure 8.9).

Oxygenated blood passes up the umbilical vein or veins from the placenta to join the portal vessels and ductus venosus in the liver. The ductus venosus provides a short-cut between the umbilical vein and the inferior vena cava. Not all fetal mammals have one, for example foals do not. The oxygenated blood passes via the ductus venosus to the vena cava, from where it is preferentially streamed through the foramen ovale in the atrial septum to the left atrium, so to the left ventricle and from there to the coronary and cartoid arteries. This wonderful adaptive mechanism can be demonstrated beautifully with modern 'colour flow' Doppler ultrasound equipment. Deoxygenated blood returning from the upper (superior vena cava) or lower (inferior vena cava) parts of the body flows into the right atrium, on to the right ventricle and into the pulmonary arterial trunk. However, little of this pulmonary arterial blood flows into the high-resistance vascular bed of the lungs; rather, the majority of the right ventricular output passes through the ductus arteriosus and down the aorta to the iliac vessels, from which arise the two

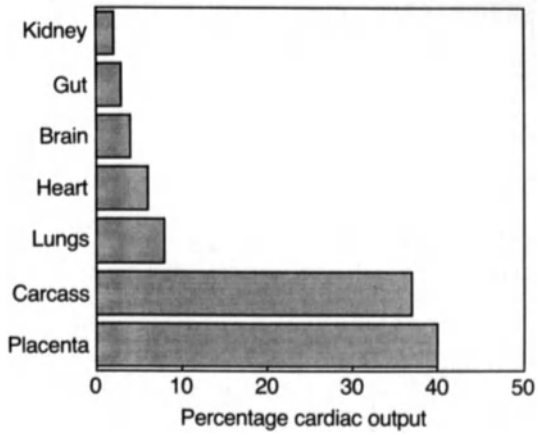

Figure 8.10 Percentage distribution of the fetal cardiac output.

large umbilical arteries which take the blood back to the placental bed. In the fetal lamb, approximately half of the combined ventricular output enters the umbilical arteries.

As a result of this anatomical arrangement, between 75 and 80% of the blood supply to the liver is from the umbilical vein, and this will be oxygenated blood rich in nutrients. Most of the remainder of the supply comes from the portal vein; in the fetal lamb, most of the portal blood goes to the right lobe of the liver; thus the liver will have a low risk for damage due to fetal hypoxia. The brain and heart receive a preferential delivery of oxygenated blood. In lambs, these organs take around 4 and 3% of the combined output respectively (Figure 8.10).

In man, the fetal brain receives 15% of the cardiac output. The lungs, on the other hand, are perfused with blood with relatively low oxygen content and partial pressure. So too are the kidneys and bowel.

(b) Hypoxia – a challenge

The mature adult heart responds to acute hypoxia by an increase in rate and cardiac output and with a redistribution of the circulation. The fetus may experience hypoxia following an interruption in oxygen supply anywhere from the mother's nose to the umbilical vein. Depending on which, the effects may be different. Experimental studies which induced hypoxia by lowering fetal arterial oxygen content, limiting umbilical blood flow or clamping the umbilical vein all caused fetal bradycardia and a reduction in combined ventricular output to some degree. Teleologically, this paradoxical response may be appropriate in so far as myocardial oxygen consumption will be reduced with the fall in heart rate and is directly comparable with the diving reflex in marine mammals. With maternal hypoxaemia or fetal anaemia, which have relatively less effect on cardiac output, the circulation to fetal heart and brain were enhanced, whilst those to gut, kidney and lung were reduced. These are the organs which are damaged, sometimes irreparably, by intrauterine hypoxia (Peeters *et al.*, 1979; Fumia *et al.*, 1984). It is noteworthy that, in the majority of fetal tissues, oxygen delivery is maximal at the relatively high haematocrits of the normal fetus (Fumia *et al.*, 1984). Cord clamping, as might be anticipated, invariably reduces cardiac output; the circulation to the brain and heart, whilst not enhanced, is maintained, but all other organs suffer.

Most of these classical experiments were performed with acute challenges. Of equal importance are the effects of chronic hypoxia, which have been studied in much less detail. However, recent experiments in the chronically catheterized sheep exposed for up to 4 days of hypoxia have again demonstrated the remarkable 'oxygen margin of safety' of the fetus compared with the mature animal (Rurak *et al.*, 1990). Initially, an imposed 40% reduction in oxygen delivery to the fetoplacental unit was compensated for by a 30% increase in umbilical blood flow. By 8h, there was a progressive metabolic acidaemia and a 70% reduction in oxygen delivery, but myocardial oxygen delivery was maintained.

The fetus has, in addition to these primary cardiovascular reflexes of bradycardia and

blood flow redistribution, many other adaptive mechanisms which help protect it against hypoxia. The higher oxygen-carrying capacity of the blood is described below. Fetal tissue oxygen extraction can increase and metabolic activity can be reduced, aided by behavioural state changes and reduced fetal movements. The human fetal liver is much larger, as a fraction of total body weight, than the adult organ, and this is partly due to extensive liver glycogen stores. Aerobic glucose metabolism is the optimum energy source for the normoxic fetus as this provides the maximum amount of ATP per unit of oxygen. During hypoxia, hepatic glycogenolysis releases large amounts of glucose to fuel the less efficient production of ATP from anaerobic metabolism (utilization of fetal myocardial glycogen is also an important factor in the fetal resistance to hypoxia). Hence, the growth-retarded fetus is much more vulnerable to asphyxia because the reserves of liver glycogen, and peripheral stored fat, have already been depleted.

There are also hormonal adaptations to fetal hypoxia (Court *et al.*, 1984; Martin *et al.*, 1987; Cheung and Brace, 1988). Infusion of adrenaline or noradrenaline into the sheep fetus results in a lowering of fetal insulin concentrations and an elevation of fetal cortisol and lactate concentrations and femoral artery PaO_2 (Hooper and Harding, 1990). Catecholamine concentrations rise in the fetus in response to both acute and chronic hypoxia, and one interpretation of these infusion experiments is that this humoral response may protect the hypoxic fetus by inhibiting insulin secretion, reducing entry of glucose into peripheral tissues, thereby sparing tissue oxygen consumption and allowing oxygen and glucose delivery to the brain to be maintained.

There are a few clinical footnotes which are relevant to this discussion of acute and chronic fetal hypoxia. Traditionally, the Apgar score, based on heart rate, respiration, muscle tone, reflex response and colour (Apgar, 1953), has been used to assess the degree of asphyxia of the human infant at birth. In theory, the more asphyxiated the infant, the worse the Apgar score, although the soundness of this correlation has often been questioned (Marlow, 1992). Umbilical arterial or venous pH has been suggested to be a more accurate marker of intrapartum hypoxia than the subjective Apgar score, but there is little evidence from longitudinal studies that cord acid–base status is a reliable predictor of brain damage (Dennis *et al.*, 1989). It seems that chronically hypoxic infants with a metabolic acidosis, who do not respond to further acute hypoxia with increasing acidosis, may be at greatest risk of subsequent handicap (Dennis *et al.*, 1989). The implication is that, in the human infant, the ability to respond to intrapartum hypoxia with acidosis, suggesting a switch to anaerobic metabolism, is protective, and this is consistent with the animal data described above. Unfortunately, however, prospective studies have not shown cord lactate concentrations to be of predictive value (Marlow, 1992). Abnormalities of the fetal heart rate pattern or morphology (loss of variability, decelerations or ischaemic changes), measured through the mother's abdomen by ultrasound or from a fetal scalp electrode, have been described as indicators of fetal hypoxia. However, monitoring the fetus during labour may not improve the outcome (D. MacDonald *et al.*, 1985). The presence of meconium in the amniotic fluid, assumed to reflect a stress response by the fetus, is an unreliable indicator of intrapartum asphyxia. In terms of active therapeutic interventions to help the fetus, as opposed to simply monitoring fetal well-being, one study has shown that in the human maternal hyperoxygenation may improve fetal outcome (F.C. Battaglia *et al.*, 1992).

(c) Control

The control of the fetal circulation by baro- and chemoreceptors, central and peripheral, and other higher nervous mechanisms has received much attention over the last three decades (Biscoe *et al.*, 1969; Purves, 1981; Hanson, 1993). The hypoxia-induced bradycardia in the late-gestation fetal sheep appears to be primarily mediated via peripheral chemoreceptors and the vagus (Walker, 1984). A qualitatively similar response to fetal hypoxia is seen in the llama but at an earlier fractional gestation, and this may represent an evolutionary adaptation in an animal which may climb to 15 000 feet altitude during the pregnancy (Benavides *et al.*, 1989).

Baroreceptors are located at the bifurcation of the carotid arteries (carotid sinus) and in the aortic arch, and vagal discharge synchronous with the fetal arterial pulse has been recorded in fetal lambs (Ponte and Purves, 1973). The maturation of the function of the baroreceptors during fetal life has been studied in the sheep fetus but, although baroreceptor sensitivity appears well developed in the newborn human infant (Long and Henry, 1992), baroreceptor reflexes exhibit a depressed sensitivity at birth and a progressive post-natal maturation to adult levels (Walker, 1984). Induced decreases in heart rate using an aortic balloon in fetal lambs were abolished by denervating the carotid sinus and stripping the aorta, suggesting a functional role. Atrial stretch receptors also appear to be competent in fetal life (Stephenson and Broughton-Pipkin, 1990) and may contribute to the regulation of blood pressure via their influences on circulating volume (Cheung and Brace, 1989). As gestation proceeds, baroreceptor sensitivity remains unchanged but baroreflex sensitivity to an increased blood pressure decreases and sympathetic tone increases, both of which may contribute to the increase in blood pressure which is seen with increasing gestation (Stephenson *et al.*, 1991). Baroreceptors also discharge in response to hypoxia (Eckberg and Sleight, 1992). The role of central neuroregulatory mechanisms in controlling blood pressure in the fetus, and their interaction with intravascular volume, remains unclear. A number of new transmitters which may be involved in this process have been investigated recently, but a full discussion is outside the scope of this review and the reader is referred elsewhere (Long and Henry, 1992). The renin–angiotensin system is active in fetal life (Stephenson *et al.*, 1991) and may contribute to control of blood pressure (Iwamoto and Rudolph, 1981).

The fetal heart responds weakly, if at all, to increased peripheral resistance, and a rise in peripheral resistance results in a fall in cardiac output. The cardiac output varies primarily with heart rate, there being much less variability in stroke volume in the fetus. Fetal tachycardia means increased output, bradycardia a decrease and a marked bradycardia causes a marked decrease in cardiac output.

8.3.2 BLOOD AND OXYGEN TRANSPORT

(a) Red blood cells

The need for an efficient oxygen transport system is paramount for the growth and development of the conceptus. The evolution of the respiratory proteins, from a relatively simple form like myoglobin to the more complex haemoglobins, allowed an increase of some hundredfold in the oxygen-carrying capacity of extracellular fluids. Initially, most mammalian species produce an embryonic and/or fetal form of haemoglobin with a higher affinity for oxygen than the adult form. This is not, however, universal, and neither cats nor pigs appear to make a 'fetal' haemoglobin (Hb_F; Delivoria-Papadopoulos *et al.*, 1974).

In the very early stages of development, precursor cells (haemangioblasts) arise from the mesenchyme and are the originators of

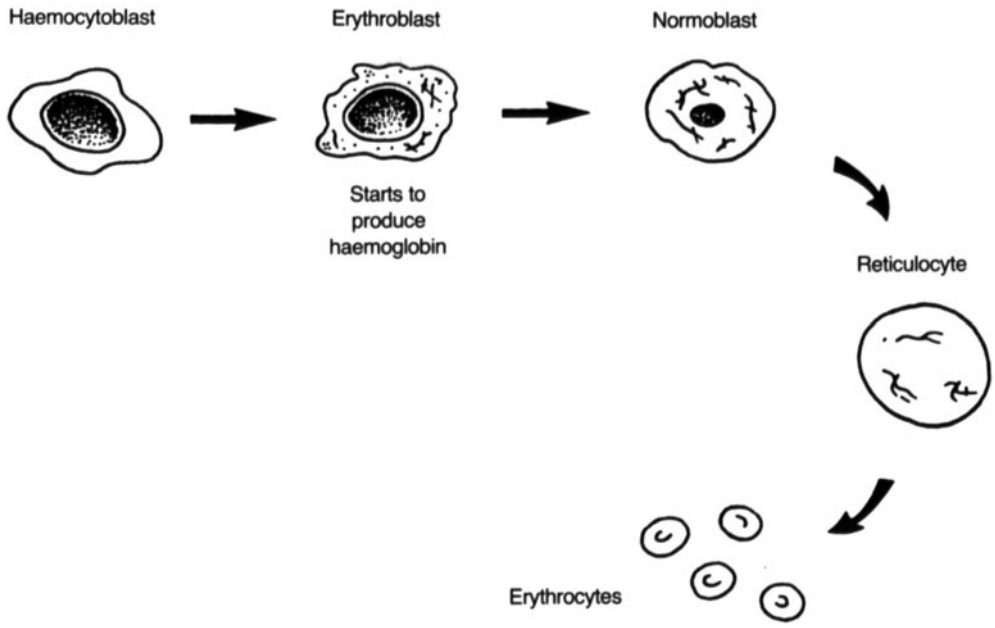

Figure 8.11 The chain of cell transformation which results in erythrocyte production. Erythropoietin acts on the haemocytoblasts to increase their rate of division and initiate the synthesis of haemoglobin in the erythroblasts so formed.

both the capillary endothelial cells and the haemoblasts (Figure 8.11).

These cells are large, basophilic and migratory. The blood islands of the yolk sac are the first fixed site of haemopoiesis and their successful development requires an interaction between the extraembryonic endoderm and the mesoderm. The blood islands themselves consist of sinusoids, full of erythroid cells, which are nucleated red cells containing embryonic haemoglobin (Hb_E). The capacity to synthesize Hb_E is evident by about 3 weeks of gestation in man, 8 days of gestation in the mouse and 13 days in the gerbil. This Hb_E has two forms in man, known as Gower 1 and Gower 2. The Gower 1 form is the most primitive, and consists of four epsilon-chains. There is rapid development of Gower 2, with two alpha- and two epsilon-chains ($\alpha_2\epsilon_2$). The structure of both alpha and epsilon chains closely resembles myoglobin, which itself has a fetal as well as an adult form (Kagen, 1973). Production of this primitive haemoglobin in the yolk sac appears not to be controlled by erythropoietin (E_p), unlike that from other sites later in development (R.J.Cole and Paul, 1966). The earliest haemopoiesis results in nucleated red cells with a relatively short lifespan, while definitive erythropoiesis requires expulsion of the nucleus.

When yolk sac erythropoiesis is at its height, the liver begins to act as a site of erythropoiesis and continues as such until after delivery. It seems likely that both yolk sac and liver are 'seeded' by migratory haemoblasts, probably with a common precursor. The erythrocytes produced are non-nucleated, and slightly bigger than adult erythrocytes (8 μm compared with 6 μm), although smaller than the yolk sac cells. It is around this time that an increasing proportion of cells are found to contain $Hb_F(\alpha_2\gamma_2$, and it seems possible that the type

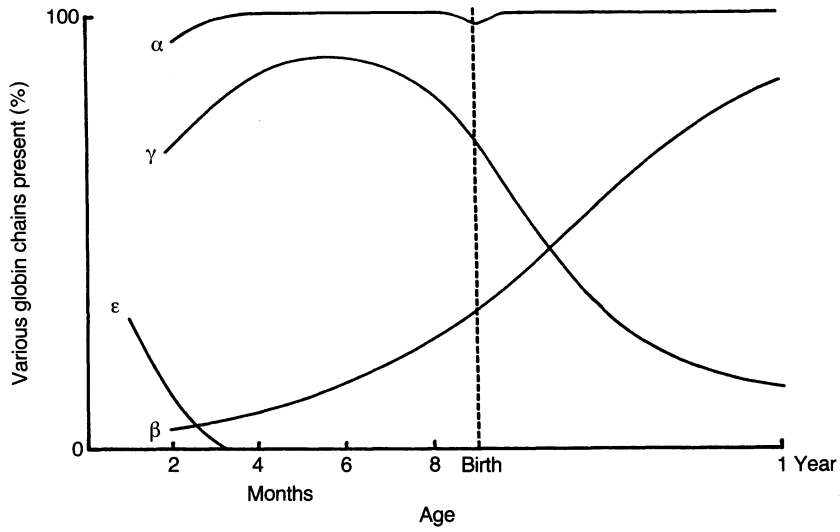

Figure 8.12 Summary to show the proportion of the various globin chains present during human fetal and neonatal life. The alpha and epsilon chains are structurally very similar to myoglobin. (Redrawn from Pearson, 1966.)

of haemoglobin produced depends on the site of production. The fetal liver can also synthesize adult-type haemoglobin (Hb_A). Although erythrocytes are the primary blood cell produced by the liver, megakaryocytes and granulocytes can also be identified in the circulation from this time and are thought to be hepatic in origin.

The spleen plays a more minor role in fetal erythropoiesis and appears to be seeded via the liver. Splenic red cell production antedates that of the bone marrow in mice and gerbils but is initiated later in man. Although hepatic and splenic erythropoiesis declines after birth, severe anaemia can stimulate both tissues again, even in adulthood (C.H. Smith, 1972).

The bone marrow, the adult site of erythropoiesis, develops late in rodents (R.A. Smith and Glomski, 1977) but is capable of some haemopoiesis from approximately halfway through gestation in man. The functional capacity of bone marrow also shows species differences, both granulopoiesis and erythropoiesis being present antenatally in man and gerbils but only granulopoiesis in the mouse.

(b) Haemoglobin

The dominant adult-type haemoglobin, Hb_A, is present in low concentration from fairly early in gestation in man but not until late gestation in other species such as the sheep (Bard *et al.*, 1978; Horvath *et al.*, 1988). Hb_A consists of two alpha- and two beta-chains ($\alpha_2\beta_2$). The beta- and gamma-chains resemble each other more closely than they do the alpha- and epsilon-chains (Pearson, 1966). It seems likely that these various globin chains have arisen by gene duplication from an epsilon or myoglobin-type ancestor. The genetics of the switching from one type of haemoglobin to another has been discussed in some detail by Stamatoyannopoulos and Nienhuis (1981). There are considerable implications for the treatment of the haemoglobiopathies if this developmental switch could be prevented (Weatherall, 1982). The proportions of the various type of globin chain produced during human gestation are summarized in Figure 8.12.

Some 15% of the haemoglobin in man is still of the fetal type by the age of 1 year, but

in other species Hb_A may be the only form present by late gestation (e.g. rats: Stein *et al.*, 1971).

Fetal muscle is deficient in myoglobin, the content in man being only approximately 1% at 5–7 months' gestation, by comparison with adult values, and 6% at birth (Kagen, 1973); other species (horse, dog, cow, pig) show similarly low values. The synthesis and function of myoglobin *in utero* do not appear to have been extensively studied.

The two major factors affecting the oxygen affinity of haemoglobin *in utero* are the Hb_F concentration and the content of the inorganic phosphate 2,3-diphosphoglycerate (2,3-DPG) in the red cells. The total inorganic phosphate content is 65% greater in fetal lambs than in adult sheep and this difference lies largely in the ATP and DPG contents (F.C. Battaglia *et al.*, 1970). Hb_F has a much lower affinity for 2,3-DPG than Hb_A, and the synthesis of 2,3-DPG is also lower *in utero* than in the perinatal period. This has the effect, summarized in Figure 8.13, of shifting the fetal oxyhaemoglobin dissociation curve to the left.

Since the maternal red cell has a somewhat greater affinity for 2, 3-DPG during pregnancy, the curves are still further separated. Thus, at blood gas tensions of 25–60 mmHg the fetal blood absorbs oxygen, and gives up carbon dioxide, more efficiently, although at the cost of somewhat lower efficiency in releasing oxygen to the tissues.

From around 0.85 of gestation the P_{50} (the partial pressure of oxygen at which 50% of the haemoglobin is saturated) rises steadily in the fetal lamb, synchronously with the appearance of increasing proportions of Hb_A in the circulation (Figure 8.14) (sheep, Bard *et al.*, 1978; man, Linderkamp *et al.*, 1984a,b).

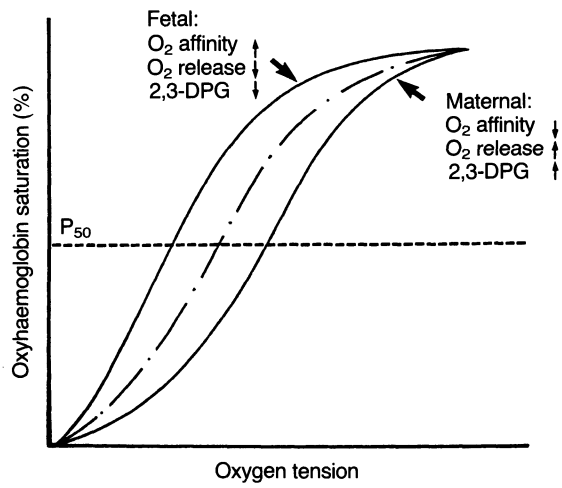

Figure 8.13 The lower affinity of fetal haemoglobin for 2,3-DPG leads to a leftward shift from the normal adult oxyhaemoglobin dissociation curve (–·–·–). The opposite is true of maternal haemoglobin during pregnancy. Oxygen and carbon dioxide are thus more easily transferred across the placenta. P_{50} = oxygen tension at 50% oxygen saturation of haemoglobin.

(c) Erythropoietin

Just as the site of erythrocyte production changes during gestation, so does the site of erythropoietin (Ep) production change (Anonymous, 1987; Hambley and Mufti, 1990). The liver is the predominant site of Ep production during intrauterine life and, even in adulthood, can be stimulated by severe hypoxia to produce 10–20% of the total circulating Ep (Zanjani *et al.*, 1977). The kidneys appear functionless in this respect until about 0.82 gestation in the lamb, after which time they synthesize an increasing proportion of Ep until some six weeks postnatal age when, under normal conditions, they become the sole site of synthesis (Zanjani *et al.*, 1981). However, in the rat, renal synthesis of Ep does not appear to be initiated until around the time of weaning (day 20) and is not of primary importance until about day 40 (Lucarelli *et al.*, 1964). This is the time when the last primitive progenitor cells disappear from the circulation and the two events may be linked. The initiation of renal Ep synthesis is coincident with the appearance of Hb_A in

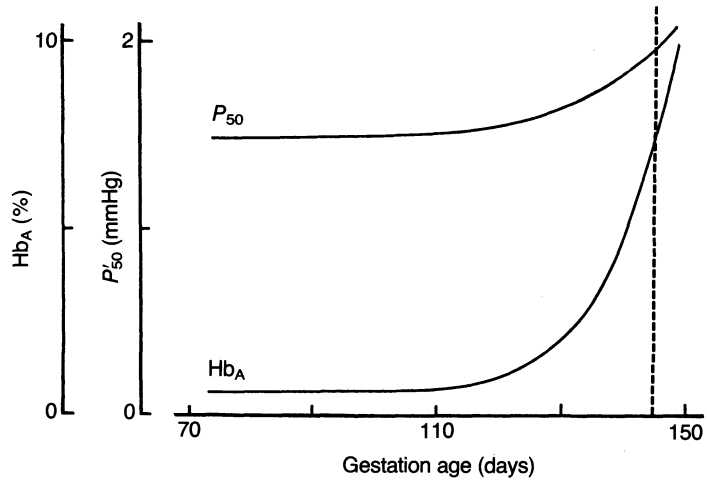

Figure 8.14 There is a temporal coincidence between the rise in P_{50} and that in the proportion of Hb_A in the circulation of the fetal lamb in the last weeks of gestation. The intracellular concentration of 2,3-DPG is unchanged over this period. (Redrawn from Bard *et al.*, 1978.)

the circulation of the fetal lamb but it is not known whether the coincidence is more than temporal.

Yolk sac erythropoiesis appears not to be stimulated by Ep (Cole and Paul, 1966), but hepatic erythropoiesis is, from early gestation in the lamb (Zanjani *et al.*, 1977) and mid-gestation in the rat (Cole and Paul, 1966). The sensitivity of the haemopoietic tissues to Ep increases to a maximum by mid-gestation. Ep acts early in the production of the red cells, acting on the haemocytoblasts to promote cell division, and commit them to haemoglobin synthesis. Once the cells are committed, they cease being sensitive to Ep. Ep appears not to cross the placenta, and fetal synthesis appears to be under local control. For example, if pregnant rats are made polycythaemic by transfusion, although maternal erythropoiesis is suppressed to zero, fetal erythropoiesis is not (Jacobson *et al.*, 1959). If, on the other hand, the mother is made hypoxic, then fetal erythropoiesis increases before maternal erythropoiesis, and to a greater extent (rats, Matoth and Zaizov, 1971; lambs, Zanjani *et al.*, 1974). This is presumably because the fetus is in a chronically mildly hypoxic state, and can respond instantly to an exacerbation of the stimulus. In the human, the infant who has experienced intrauterine growth restraint is more likely to be born with a higher haemoglobin concentration (Jones and Roberton, 1986), presumably because of the confounding variable of chronic hypoxia in association with placental nutritional failure. Macrosomic infants born to a diabetic mother also tend to have higher packed cell volumes (Lemons *et al.*, 1981), suggesting that fetal hyperglycaemia or hyperinsulinism, or other tissue growth factors, can stimulate either Ep production or the bone marrow cells directly.

Plasma Ep concentrations rise rapidly at birth, presumably under the stimulus of birth hypoxia, and may be several-fold that of the mother (Tchernia *et al.*, 1981). In the rat, even 2 h after birth, plasma concentrations of an Ep-like substance have risen significantly by comparison with those measured at delivery (Nagel and Nagel, 1984). In species immature at birth Ep concentrations remain high during suckling (e.g. mice, Meberg *et al.*, 1980;

rabbits, Holter *et al.*, 1987), whereas in those more mature Ep concentrations fall sharply after birth (lambs, Zanjani *et al.*, 1977).

(d) White blood cells

A few granulocytes and megakaryocytes are produced in the yolk sac blood islands and there is a progressive rise in both cell types from the time at which the liver takes over haemopoiesis (C.H. Smith, 1972), increasing further in mid-gestation as medullary haemopoiesis begins.

Lymphocytes are first demonstrable in the embryonic thymus (Ackerman, 1967), but these thymic colonies can rapidly disseminate during embryonic life and lymphocytes are found in blood, spleen, lymph nodes and the gastrointestinal tract during the first quarter of gestation. The thymic cells are epithelial in origin, and follow two lines of differentiation: a limited period of lymphoblast proliferation and the formation of reticuloepithelial cells. The lymphocyte content of bone marrow rises significantly between 25 and 30 weeks' gestation in man to some 25% of the total marrow cells (Yoffey and Thomas, 1964; Kalpaktsogrou and Emery, 1965). Changes in lymphocyte proportion are the only major changes in the human fetal differential cell count in the third trimester rising from 2 to 8% between 26 and 30 weeks' gestation (Kalpaktsogrou and Emery, 1965; Forestier *et al.*, 1986).

Studies of lymphocyte type and function in neonatal human blood revealed a lower proportion of both mature T cells and helper cells, together with uncommitted cells (Solinger, 1985), the proportion of which fell sharply from 32% at 24 weeks' gestation to 0% at term. It is possible that these uncommitted cells may differentiate in an extrathymic site and migrate to the spleen, bone marrow or other sites. Although there is a good mixed lymphocyte reaction at birth, cell-mediated lympholysis is poor (Solinger, 1985). Marodi *et al.* (1984) studied granulocyte and monocyte function in neonatal blood. Granulocyte function appeared mature and the oxygen consumption and hydrogen peroxide production were normal in the monocytes, indicating normal functioning of the oxygen-dependent microbicidal systems of the cells. However, there was a considerably diminished capacity to phagocytose and kill group B streptococci and *Staphylococcus aureus*. This diminished ability may partly account for the susceptibility of the newborn to infection.

(e) Platelets and clotting

Platelet counts are not markedly different in the fetus in the second half of gestation compared with adults (e.g. Forestier *et al.*, 1986). Platelet function is, however, somewhat diminished by comparison with adults, especially in relation to response to proaggregatory agents such as adrenaline (Mull and Hathaway, 1970; Ts'ao *et al.*, 1976; Andrews *et al.*, 1985). The lower response to adrenaline is associated with a lower number of adrenoceptor binding sites (C.R. Jones *et al.*, 1985) and may be a protective mechanism in the face of the extremely high plasma adrenaline concentrations found following vaginal delivery in man and other mammals.

There are also some differences in the clotting cascade, especially in relation to the vitamin K-dependent clotting factors (II, VII, IX and X), which are between 30 and 70% of adult values at full-term delivery, and even lower when studied in preterm human infants (Glader and Buchanan, 1976). The other clotting factors are either unchanged or marginally lower. Even well term infants, especially if breast fed, are at risk of cerebral bleeding or death from haemorrhagic disease of the newborn as a result of this relative immaturity of the clotting system (McNinch and Tripp, 1991). Both the partial thromboplastin time (testing the intrinsic clotting system) and the prothrombin time (testing

the extrinsic clotting cascade) are increased in the newborn, and increased further after pre-term delivery (Glader and Buchanan, 1976).

(f) Blood viscosity

The whole blood viscosity rises throughout the last third of gestation, with parallel increases in haematocrit, fibrinogen and total protein concentrations although adult viscosity has not been reached by term. When tested at low shear rates, the whole-blood viscosity of adult blood, adjusted to a 'fetal' haematocrit of 60%, was 90% greater than that of very pre-term human infants (Linderkamp *et al.*, 1984b). The difference became less at higher shear rates. The increased blood viscosity at low shear rates is a consequence of erythrocyte aggregation caused by bridging of fibrinogen molecules between cells (Linderkamp *et al.*, 1984a). Since fibrinogen concentrations are decreased before birth, the lesser aggregation would be expected. This is obviously of physiological benefit to the fetus with its low vascular resistance and perfusion pressure. But when human infants are delivered prematurely, the same poor red cell aggregation could predispose to intracranial haemorrhage.

The deformability of red blood cells is another important determinant of oxygen transport and release to the tissues in the microcirculation. Fetal and neonatal red cells do not differ from adults in their deformability on testing *in vitro* (Linderkamp *et al.*, 1986). Their filterability may, however, be less, and this may in part account for their somewhat shorter lifespan. The maternal llama has elliptical red cells, presumably an adaptation which is favourable during chronic hypoxia at high altitude. Although Sir Joseph Barcroft likened the challenges faced by the fetus to those of the mountain climber, this unusual type of corpuscle has certainly not evolved in the human fetus although spherocytes (rather than the mature biconcave form) are not uncommon (O'Callaghan and Stephenson, 1992).

8.3.3 LUNG DEVELOPMENT

(a) Anatomical development

In man, lung growth and development begins early in gestation and continues into childhood. Hence, disturbances *in utero* and in early childhood (e.g. congenital abnormalities, maternal smoking during pregnancy (Young *et al.*, 1991) may have profound effects on the subsequent lung growth and function of the child (Hodson, 1992). At about 4 weeks after conception, the lungs begin as a bud arising from the foregut; this divides and branches to form the lining of the respiratory tract. Bronchial budding continues with further subdivisions so that three-quarters of bronchial branching is achieved by the 10–14 weeks and the process virtually complete by 16 weeks (Helms, 1992). The canalicular period from 17 to 26 weeks' gestation represents the first appearance of potential gas-exchange surfaces (Boyden, 1977), during which alveoli appear and develop in both complexity and surface area. Not surprisingly, there is an exponential increase in the mortality due to pulmonary immaturity for infants born within this gestational age window. At birth, the newborn human infant possesses terminal air spaces, sufficient for initial gaseous exchange. However, only after birth do alveoli form and develop to the state found in adults. Splanchnic mesoderm envelops the dividing bud to form the supporting structures. In the upper airways these are the surrounding muscle and cartilage; in the lower they form the matrix of elastin and collagen which determine the lungs' performance characteristics. It also provides the other interface of the gaseous exchange, the pulmonary capillaries and circulation (Hislop and Reid, 1981).

(b) Lung growth

Over the second half of gestation, the fetal lung grows at a rate similar to the body as a whole, whether expressed as wet or dry weight or DNA content. Physical factors have a powerful influence on the growth of the lung whilst hormonal factors modify its rate of maturation. The major physical factors include: the space in the thorax, the volume of amniotic fluid, fetal breathing and airway distending pressure (Kitterman, 1986).

Thoracic cage volume

It has been known for many years that babies born with ill-formed diaphragms, such that the bowels in part develop in the thoracic cage (diaphragmatic hernia), have small hypoplastic lungs, and it is this consequence of the disorder which is often lethal. This effect has been mimicked experimentally in fetal animals. For example, Harrison *et al.* (1980) placed an inflatable balloon into the left thoracic cage of a fetal lamb and were able to demonstrate that when it was inflated the lung ceased to grow but when the balloon was deflated it began to grow again. So, for the lung, the size of the container determines its growth, unlike the brain in its distensible box. Under normal circumstances, the junctions of the cranial vault only seal when the brain stops growing.

Adequate amniotic fluid

The lungs of the term fetus are not collapsed. The potential airways are filled with about 30 ml/kg body weight of lung liquid, which is secreted by the pulmonary epithelium (Jobe, 1984) (for comparison, the tidal volume of air in a newborn infant is 6–10 ml/kg). The clinical correlation between chronic amniotic fluid leakage (and hence oligohydramnios and failure of lung liquid volumes to be preserved) and pulmonary hypoplasia is well recognized. Once the fetal skin becomes impermeable to water (section 8.3.8), the kidney is the main source of amniotic fluid. If the kidneys are ill formed and no urine is produced (renal agenesis), then there is very little amniotic fluid. This is usually, if not invariably, associated with hypoplasia of the lungs. In experimental studies, fetal urine release was stopped in rabbits by tying the ureters (in lambs by removing the kidneys). This produced oligohydramnios and lung hypoplasia but the results were not always clear cut. Tapping the amniotic space in early human pregnancy (amniocentesis) and drawing off fluid also leads to lung hypoplasia. The results are variable and may depend on the time of the procedure in the pregnancy and whether the membranes continue to leak. This has been mimicked experimentally in guinea pigs, and it has been shown that even a short period of oligohydramnios interferes with lung development (Moessinger *et al.*, 1986). In this species, the extent of this interference depends to a large extent on the time of onset (greater impact during canalicular stage than terminal sac stage) and to a lesser extent on the duration of oligohydramnios. Oligohydramnios in the fetal guinea pig for a period as short as 8% of gestation led not only to a significant decrease in lung volume (and the surface area available for gas exchange) but also to pulmonary structural alterations. The hypoplastic lungs contained fewer saccules (fetal 'alveoli') and the amount of elastic tissue per unit volume was reduced (Collins *et al.*, 1986). So it does seem that loss of amniotic fluid produces the lung hypoplasia. The question is how. It might be secondary to limitation in movement and compression of the thorax because of the loss of liquid (oligohydramnios) or to a fall in tracheal distending pressure, or a variable combination of these and other factors.

Fetal breathing movements

The nature of fetal 'breathing' movements will be described later (section 8.3.3d). There

is some evidence that integrity of the nervous control of the diaphragm, and therefore fetal breathing movements, is important in lung growth. In human infants, paralysis of the diaphragm at birth is rare but does occur; in fetal lambs phrenic nerves have been deliberately cut. In both situations the lungs are hypoplastic. However, it could be argued that the paralysed diaphragm ballooned upward and exerted its effect by reducing thoracic space, and it is this effect, rather than the cessation of breathing movements, which resulted in lung hypoplasia. However, cutting the cervical cord above the phrenic nuclei appears to leave the diaphragmatic tone intact but abolishes breathing movement. In this situation, lung hypoplasia occurs, indicating that it is the breathing movements which are important (Wigglesworth and Desai, 1979).

Pressure in the potential airways

If the trachea of the fetus is occluded by a ligature the lungs become distended (Jost and Policard, 1948). The lungs secrete fluid and the laryngeal sphincter influences its flow into the amniotic space. Experimental partial obstruction of the respiratory tract increases lung growth; chronic drainage of lung liquid has the opposite effect. Thus, a modest airway pressure may act as a stimulus to lung growth, and this will be determined by the rate of lung liquid secretion and laryngeal sphincter activity. For the purpose of description, the physical factors have been discussed in isolation but it is obvious that they are inter-related.

(c) Lung liquid

The lungs secrete a liquid, an activity which appears to be essential for lung growth. The volume of lung liquid at any moment is 20–30 ml/kg in the lamb in the last trimester of pregnancy (the net flow from the lungs into the amniotic cavity is around 500 ml/day) and this traffic results in an intratracheal pressure around 2 torr, a distending pressure. The flow is episodic and reflects fetal breathing movements (Adamson *et al.*, 1973). Transient negative deflections in intratracheal pressure are seen with fetal breathing movements, due to downward movement of the diaphragm, and these are associated with 'inspiration' of a small volume of fluid, but over a period of time there is a net efflux of lung liquid into the amniotic cavity. If the pressure increases, the lung grows more (Alcorn *et al.*, 1977). It also matures faster, developing more surfactant-producing cells (type II pneumocytes). The opposite is also true (Olver, 1983; Bland, 1986).

Lung liquid is derived from the pulmonary circulation. Water from the pulmonary capillary passes first to the interstitial space across the endothelial barrier and then into the respiratory tree across the pulmonary epithelium. Strang and colleagues worked out some of the rules of this transfer (Olver and Strang, 1974). Lung liquid is low in protein and bicarbonate, high in chloride, and has a relatively low pH. It is the active transfer of chloride across the pulmonary epithelium, the chloride pump, which draws the water out of the protein-rich interstitial space. Altering pulmonary perfusion, blocking respiratory breathing and various drugs and hormones have relatively small effects with the exception of beta-adrenergic agonists (see below).

At birth the lung liquid has to be cleared to make way for air. It was thought that the fluid was cleared, in part, by flowing out of the upper airway once the head emerged from the birth canal and, in part, by lung lymphatics and circulation after birth. However, there is reasonable evidence in experimental animals (Kitterman *et al.*, 1979) and in man (Strang, 1977) that:

1. The rate of lung liquid secretion falls and virtually stops by the moment of birth.
2. Some of the lung liquid has already been

cleared out of the potential air space by the pulmonary circulation, that is the process is reversed.

Cortisol may be involved in the former and beta-adrenergic agents in the latter.

An increased risk of respiratory symptoms following Caesarean section is well recognized in the human newborn (Tudehope and Smyth, 1979; Cohen and Carson, 1985). The effect of mode of delivery on lung expansion at birth has been examined, and a striking difference between vaginal delivery and delivery by elective section was that the babies in the latter group were more likely to fail to retain air in the lungs at the end of the first breath (Vyas, 1986). In contrast, babies delivered by emergency Caesarean section, following the onset of spontaneous labour, all achieved an immediate functional residual capacity. The differences between the vaginal delivery and emergency Caesarean section babies is partly due to the fact that about one-third of the fetal lung fluid is removed by the squeeze of the baby's chest during passage through the birth canal (Greenough *et al.*, 1992). The differences between the babies delivered by elective Caesarean section and emergency Caesarean section are likely to be due to a combination of the squeezing of the thorax within the uterus (especially if the membranes have ruptured) and the exposure of the fetus to an adrenaline surge.

(d) Fetal breathing movements

If one were asked, knowing nothing about the subject, whether the fetus would flex its diaphragm prior to birth, for otherwise it would have to start to contract rhythmically at the moment of birth and continue without fail until death, then it would seem obvious that it would. If one then asked the question 'how could it work, knowing that the lung was filled with fluid rather than air?', then the problem becomes more interesting. Add to that the question of whether such performance would be reflex or under higher cortical control and then place that within a developmental scale and it is not difficult to appreciate why it has fascinated some of the finest fetal physiologists (see review Dawes, 1986). Leonardo da Vinci thought that the fetus did not breathe because, if it did, it would drown. However, from the time of Vesalius, fetal breathing movements have been observed indirectly, and from the 1970s fetal breathing has been visualized directly in human pregnancy by ultrasound scanning. In lambs, fetuses also have intermittent periods of rhythmic breathing movement, and this has been observed mostly in the second half of gestation. Breathing movements are irregular in rapid eye movement (REM) sleep but become regular in deep (non-REM) sleep. Breathing movements may be important in lung growth and may well be critical for post-natal performance. In the sheep fetus, the amount of breathing decreases as birth approaches and then must change to a continuous pattern following parturition.

There is no doubt that fetal breathing movements reflect fetal well-being and are influenced by hypoxia and hypercapnia and are modulated by neurotransmitters and neuropeptides, often in ways contrary to those which occur after birth. However, fetal breathing is discontinuous and, because of this and conflicting studies in the literature (Blott *et al.*, 1987; Moessinger *et al.*, 1987), the use of fetal breathing movements as an index of fetal health in clinical practice is limited.

(e) Lung maturation

When they speak of the process of lung maturation, authorities seek to comment on function more than structure and, to a certain extent, maturation can be dissociated from growth. Thus, a lung may be small but mature. That is, it has the right balance of structural material and epithelial cells to permit lung expansion with air and the maintenance

of a functional residual capacity. One key element of this, which has received considerable attention, is the secretion of a natural detergent, surfactant, by the pneumocyte type II cell (Comroe, 1977). By contrast, some large lungs may have delayed maturation and insufficient surfactant and these present the newborn with breathing problems after birth. An example is a diabetic infant born by elective Caesarean section at term. Some of these maturation phenomena are associated with birth induction and are accelerated during the birth process.

Mechanisms involved include glucocorticoids, which have been used to induce maturation in infants who are threatening to be born prematurely (Dancis *et al.*, 1980; Crowley *et al.*, 1990; Levene, 1992). Cortisol, prolactin and triiodothyronine appear to act synergistically in accelerating lung maturation in fetal sheep (Liggins, 1990). Neither prolactin nor triiodothyronine crosses the human placenta in significant amounts, thus ruling out the option of fetal therapy via maternal administration. However, thyrotrophin-releasing hormone, a small peptide, does cross the placenta, and the results of further clinical trials are awaited (Morales *et al.*, 1989). It is the beta-receptor agonists which are released during the birth process which have the most immediate and powerful effect, stimulating surfactant release and manufacture as well as reducing the secretion rate of lung liquid (Liggins, 1990).

(f) Pulmonary circulation

Owing to the central communications between the circulations in fetal life, there are, effectively, large intracardiac (through the foramen ovale) and extracardiac (through the ductus arteriosus) right-to-left shunts. *In utero*, the two chambers of the heart work in parallel, the pulmonary bed taking less than 10% of the combined output (Teitel, 1992). This is not because the vasculature is meagre and narrow, but because the vessels are permanently and strongly constricted. This is achieved by generous muscles in the walls of the arteries and arteriole. The tone is maintained partly by the low fetal PO_2 (A.I. Hyman *et al.*, 1975; Alpan and Glyman, 1990) and fluid-filled unexpanded lungs. Expanding the lungs with nitrogen causes a fall in pulmonary vascular resistance; it falls further if they are expanded with oxygen. With the fall in resistance, there is an increase in flow and the sequence of events essential for lung gaseous exchange and oxygen supply is set in train (section 8.4.2).

8.3.4 KIDNEY DEVELOPMENT AND WATER BALANCE

The fetal kidneys in late gestation appear to be the primary source of the amniotic fluid (D.P. Alexander and Nixon, 1961; Houston and Zeis, 1976) but are not necessary for volume regulation or nitrogen excretion since the placenta is an extremely efficient dialysis unit. A fetus can survive to term without kidneys, but the deficient amniotic fluid results in pulmonary hypoplasia, and multiple skeletal and cartilaginous anomalies due to it being 'squashed' within the uterus (Potter, 1946).

The ionic and osmolal characteristics of the extracellular fluids of all the vertebrates are very similar, and suggest that our common ancestor lived in brackish water. The transition from the sea into brackish or fresh water with its much lower osmolality, and subsequently to dry land, demanded the development of regulatory mechanisms to avoid on the one hand the cell swelling with rupture and on the other desiccation. There also developed the need to protect the embryonic stages of these adventurous species from being osmotically disrupted. The parallel development of the genital and urinary tracts subserved both these functions, and this evolution is recapitulated during mammalian fetal development.

Another requirement imposed on the creatures who left the water was the need to reorganize nitrogen metabolism so that the ammonia resulting from protein metabolism could be converted into other metabolites which, unlike ammonia, are relatively nontoxic. The mechanisms for nitrogen excretion and water conservation developed together.

(a) Structure

Pronephros

The pronephros, the most primitive form of vertebrate kidney, develops from a mesh of capillaries originating from the abdominal aorta and functions at a perfusion pressure of more than half that in the aorta (see Brod, 1973). This results in a plasma ultrafiltrate which drains into open-ended primitive kidney tubules (nephridia) where reabsorption of individual electrolytes takes place. Pronephroi are developed in the mammalian fetus, but are only present transiently in very early gestation. Before the pronephroi degenerate, they have sent paired collecting ducts caudally, to empty into the cloaca. These structures are essential to the subsequent development of the meso- and metanephron (Gruenwald, 1952).

Mesonephros

Mesonephroi first appear in fishes, and in lower vertebrates they serve as the major excretory organ throughout life. The mammalian mesonephros, which is present only in fetal life, shares many features with the adult mesonephros in fish, particularly in relation to the degree of glomerular and tubular development and the sinusoidal nature of the tubular circulation (Davies, 1952). It seems primarily adapted to life in an aquatic environment, where water conservation is not a problem. This limitation may be the reason for the evolution of a third type of kidney, the metanephros, programmed for water conservation as well as the maintenance of ionic and osmolar balance and nitrogen excretion.

Table 8.11 The mesonephroi of different mammalian species appear at different periods of development and are present for differing periods of time. The proportion of total gestation at which the organs develop and regress is shown. Data from: Bremer, 1916; Kaplan and Friedman, 1942; Leeson and Baxter, 1957; de Martino and Zamboni, 1966.

Species	*Gestation length (days)*	*Mesonephroi present*
Rat	22	Vestigial
Rabbit	31	0.35–0.74
Guinea pig	68	0.36–0.41
Pigs	114	<0.25–0.71
Sheep	147	0.12–0.38
Man	**280**	**0.08–0.40**

In fetal mammals, the mesonephroi develop from thoracic and lumbar mesoderm in the nephrogenic cord as the pronephroi degenerate. The general development appears to be similar in all mammals, but the relative stage of gestation at which the mesonephros appears (Table 8.11) and its apparent functional importance vary widely.

The mesonephric tubules differentiate into proximal and distal segments. The proximal tubules have columnar epithelium, characteristic of secretory function, and show a form of brush border which stains with periodic acid–Schiff (PAS) and contains alkaline phosphatase, which is an indicator of the functional ability to transfer solutes across cell boundaries (Davies, 1952; Leeson and Baxter, 1957). The distribution of alkaline phosphatase seems linked to areas of rapid growth and differentiation. The cuboidal cells of the distal tubules have neither brush border nor alkaline phosphatase but contain glycogen like the longitudinal mesonephric duct (Wolffian duct), into which they all drain, and the ureter. No loop of Henle is present in mesonephroi (Kaplan and Friedman, 1942; de Martino and Zamboni, 1966). The longitudi-

nal mesonephric duct persists into adult life in the male, as the ductus deferens, but disappears almost completely in the female.

The medial end of the primitive mesonephric tubule develops into a flattened concave disc, which gradually deepens into the form of a cup. The outer wall becomes the parietal epithelium of Bowman's capsule, while the inner wall extends over the differentiating capillary loops and becomes the glomerular epithelium, rich in endoplasmic reticulum (see Kazimierczak, 1971). Until relatively recently, it was thought that the glomerulus developed by the action of formed capillaries invaginating Bowman's capsule, but it is now generally accepted that glomerular capillaries probably develop *in situ* from mesenchymal cells which have migrated into the cleft of the S-shaped tubules (for references see Kazimierczak, 1971). These glomerular capillaries become covered with cuboidal epithelium (see above) and progressive connection to the primitive dorsal aortae proceeds from head to tail (Davies, 1952).

Although mesonephroi are structurally similar in mammalian fetuses, their functional capacity and duration of existence (Table 8.11) are not. Davies (1952) suggested parallels between mesonephric and allantoic development, the relative rates being primarily dependent on placental permeability. Thus in pigs, with epitheliochorial placentae, which may present more of a barrier to placental transfer of all but the simplest substances, the mesonephros is large and persistent, and excretes large volumes of fluid into a large allantoic sac. Sheep, also with epitheliochorial placentae, have smaller, less persistent mesonephroi, and a smaller allantois, while man, with a very permeable haemomonochorial placenta, has a minimal allantois and a small, non-functional, short-lived mesonephros. Rats and mice, with haemoendothelial placentae, have only vestigial mesonephroi (Zamboni and Upadhyay, 1981) and allantois. There is, however, an exception to this interesting concept in the rabbit, which, with a haemodichorial placenta, has a functional mesonephros present for some 40% of gestation (Table 8.11) and a small allantois which is restricted to the placental surface area.

The sheep mesonephros is functional by day 18 of gestation, and it is at this time that the allantois, which has been present as a small, almost empty, sac begins to fill up (Davies, 1952). In the rabbit, a functional mesonephros is not present until day 13 (Leeson and Baxter, 1957). In the pig, in early fetal life, the mesonephroi form up to 20% of the total fetal weight and secrete large volumes of fluid (Stanier, 1960). The efferent arterioles of the porcine mesonephros form a network of capillaries over the mesonephric tubules, suggesting a capacity for reabsorption even though the loop of Henle is absent. However, bladder fluid in the pig is iso-osmotic with serum, although that in the allantois is hypo-osmotic (Stainer, 1960). It appears that the chorioallantois in the pig can reabsorb sodium and presumably chloride from at least 0.4 of gestation (McCance and Widdowson, 1960; Stanier, 1960). Fructose is also accumulated in increasing concentrations in allantoic fluid in the fetal lamb in early gestation, although in the amniotic fluid the concentration is only about one-tenth (Davies, 1952). The reason for this accumulation is not known.

The marsupials are in many ways anomalous, but it is interesting that the American opossum *Didelphis virginiana*, whose young are born after only 12½ days' gestation, has mesonephric kidneys at birth which persist until the 10th post-natal day and appear to provide the primary excretory capacity for the newborn during that time (Krause *et al.*, 1979).

Metanephros

Although structurally similar, the metanephroi initially develop their collecting

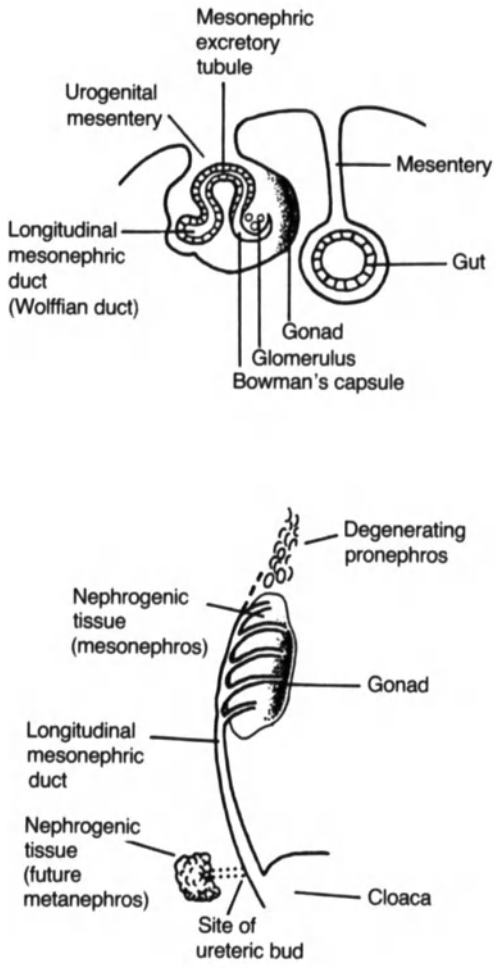

Figure 8.15 Top: Representation of a transverse section through the lower thoracic region of a 35-day human fetus, showing development of the mesonephros and its association with the future gonad. Bottom: Longitudinal section through the abdominal region of the same fetus.

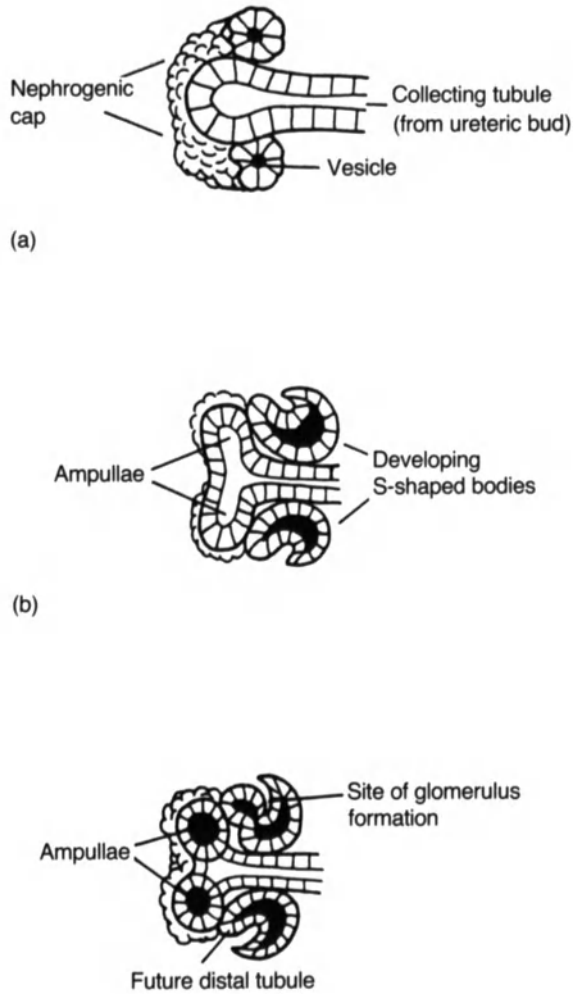

Figure 8.16 Stages in nephron development in the mammalian metanephros. The collecting tubule system arises from the ureteric bud (see Figure 8.15) and not *de novo* as in the mesonephros. (Redrawn from Kazimierczak, 1971.)

ducts in a substantially different fashion from the mesonephros.

As the mesonephros starts to regress, a ureteric bud grows out from the mesonephric duct close to its junction with the cloaca (Figure 8.15) and grows cephalad into the most caudal nephrogenic mesoderm, which forms a nephrogenic cap over the blind end (Figure 8.16).

This blind end dilates and subdivides, forming first the major and subsequently the minor calyces. The mesoderm forms condensation vesicles around these ampullae. The vesicle walls grow differentially and form the characteristic S-shaped bodies of very immature glomeruli. The 'tail' closest to the ampulla eventually fuses with it, forming the distal convoluted tubule; the middle section,

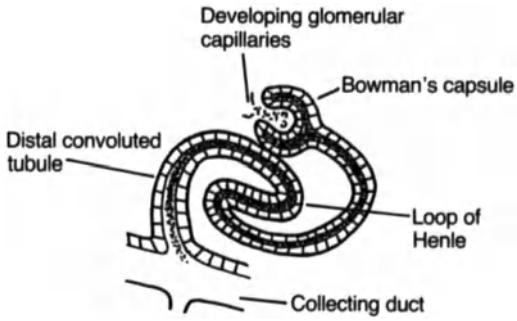

Figure 8.17 Development of the glomerulus and proximal and distal tubules of the metanephros.

as it lengthens, forms the loop of Henle and the proximal convoluted tubule, while the proximal end of the nephron forms the cup-shaped invagination (Bowman's capsule) within which the glomerular capillaries form (Figure 8.17) (Aoki, 1966).

The early calyces themselves subdivide, and this process is repeated until some 12 generations of tubules are present, with their associated nephrons. In any species, the juxtamedullary nephrons are, thus, most mature at any time during gestation, and glomerular maturation proceeds centrifugally. This pattern of development, and the state of maturity at birth, determines renal function at birth and in the neonatal period.

The renal blood supply also develops centrifugally, with the arcuate arteries forming the first major subdivision after ramification of the renal artery. Indeed, the only arteries in the developing nephrogenic cortex of pigs and rats at the time of birth are the arcuate arteries (Kazimierczak, 1971), with interlobar and interlobular branches in the more mature cortex. These latter give rise to the afferent arterioles which run along the prospective distal tubules of the more mature glomeruli. The efferent arteriole originates from the capillaries formed within the glomerulus and becomes continuous with the peritubular capillary plexus in the more mature nephrons.

One of the differences between meso- and metanephroi is that a juxtaglomerular apparatus is developed in the more cortical nephrons of metanephroi at the hilum of the glomerulus, where the early distal tubule runs in close apposition to the afferent arteriole. This is a relatively late development in glomerular differentiation, and has been described in detail by Kazimierczak (1971) in fetal pig, rat and human kidneys. It appears that the juxtaglomerular apparatus, with its intimate associations with the renin–angiotensin system, has developed to allow the fine tuning of sodium balance. Conditions which result in increased renin synthesis and release from the macula densa, with consequent afferent arteriolar vasoconstriction in the more cortical glomeruli, will increase the proportion of the intrarenal blood flow passing through the juxtamedullary nephrons, which have a higher capacity to reabsorb sodium. The development of the loop of Henle and the juxtaglomerular apparatus is thus linked to the ability to regulate salt and water balance within narrow limits, an absolute requirement for terrestrial life, whether emergence has been from brackish lagoons or from the amniotic fluid.

A pressor substance, believed to be renin, is present in pig mesonephroi (Kaplan and Friedman, 1942), but not in the mesonephroi of man (de Martino and Zamboni, 1966), cat or sheep (Tiedemann, 1976). Pig mesonephric renin is not present in specific granular cells, and this discrepancy has also been noted in the metanephroi of fetal rats near term (Tsuda *et al.*, 1971). In rats, nephrogenesis is not complete until after birth (Cain and Krause, 1971; Goncharevskaya and Dlouha, 1975; Table 8.12).

Renin has also been identified in human fetal kidneys before Bowie-positive granules are identifiable (Molteni *et al.*, 1974). Cells containing renin granules can be present before macula densa differentiation has occurred (Dhiab *et al.*, 1981); their association with the adult-type juxtaglomerular apparatus is thus a late phenomenon.

Table 8.12 Nephrogenesis is completed at widely differing rates in different mammalian species. The timing is shown as a proportion of the length of gestation, thus in those species in which nephrogenesis is incomplete at birth, a figure of more the 1.0 will be found. Data from: Potter and Thierstein, 1943; Leeson and Baxter, 1957; MacDonald *et al.*, 1959; Spitzer and Edelman, 1971; Broughton-Pipkin, 1973; Goncharevskaya and Dlouha 1975; Merlet-Benichou *et al.*, 1977; Friis, 1980.

Species	*Age at completion*	
Rat	10 days post-natal;	1.45
Rabbit	17 days post-natal;	1.55
Guinea pig	55 days gestation;	0.81
Pig	21 days post-natal;	1.19
Sheep	128 days gestation;	0.87
Man	**35 weeks' gestation;**	**0.88**

(b) Function

The study of renal function before birth is not easy. Measurements of fetal fluid volume and electrolyte composition have been made for many years in anaesthetized laboratory or freshly slaughtered domestic animals (e.g. rabbits, McCance and Stainier, 1960; guinea pigs, Boyland *et al.*, 1958; pigs, McCance and Stanier, 1960; McCance and Widdowson, 1960; sheep, D.P. Alexander *et al.*, 1958; rhesus monkeys, Chez *et al.*, 1964) but these were, at best, static measurements. The realization that electrolyte and water absorption could take place across bladder and amniotic membranes, thus modifying the composition of contained fluids, was rapidly followed by techniques for measuring function *in utero*, initially in acute experiments and more recently in chronic preparations. Indeed, the work of Mellor and Slater (1971) showed, beyond doubt, the very marked effect of anaesthesia and surgery in the fetal lamb on amniotic and allantoic fluid composition, and in an increased variability in maternal and fetal plasma electrolyte concentration. Thus, many of the data obtained under anaesthesia must be viewed with caution. Longer term experiments are, at present, technically impossible for the smaller species, and the fetal lamb has come to be used very widely in studies of fetal renal function, as indeed it has for the development of other organ systems. This is, in some respects, unfortunate since there appears to be considerable species variations in maturation of intrauterine renal function. However, in the discussion which follows, data will primarily relate to the fetal lamb, with mention made of other species as available. Some information gained in the immediate neonatal period in premature human infants will be mentioned, but should not be considered representative of normal maturation for the reasons outlined in the Introduction.

Glomerular filtration

The first stage in urine production requires ultrafiltration of plasma. The glomerular filtration rate (GFR) is substantially lower in the fetus than in the newborn, both in absolute terms and when expressed relative to body weight, body surface area, extracellular fluid volume or kidney weight (e.g. Boyland *et al.*, 1958; D.P. Alexander and Nixon, 1962; Robillard *et al.*, 1977; Alt *et al.*, 1984). There are both anatomical and functional reasons why this should be so: total glomerular surface area is less since individual glomeruli are smaller (Fetterman *et al.*, 1965; Merlet-Benichou *et al.*, 1981) and their absolute number per gram of kidney tissue is less, with nephrogenesis continuing in some species for days, or even weeks, after birth (Table 8.12); the glomerular capillaries are still morphologically immature, with a small pore size and relatively low water permeability (Vernier and Birch-Andersen, 1962); renal perfusion pressure is lower as a consequence of the low fetal arterial blood pressure (see Dawes, 1968; Gomez *et al.*, 1984) and the fetal haematocrit is usually higher than the adult (see Dawes, 1968), although this may not be so in the pig (A.A.

MacDonald, 1983). Other features influencing GFR are the colloid osmotic pressure, which is lower in fetus than newborn (Baum *et al.*, 1971), and a considerable degree of intrarenal vasoconstriction. This latter may well be hormonally mediated, possible through local generation of angiotensin II, since it decreases too rapidly after birth (Guignard, 1982) to be accounted for in anatomical terms. The renin–angiotensin system appears to be hyperactive in the newborn of all species so far studied (see Broughton-Pipkin and Symonds, 1984), and the early development of renin-containing granules in cells in apposition to the developing renal arterioles (see above) suggests a role in the regulation of intrarenal blood flow.

The renal plasma flow (RPF) is difficult to measure *in utero* since *p*-aminohippurate, the most commonly used marker molecule, is less well filtered and secreted by the immature kidney (Calcagno and Rubin, 1963; B.R. Cole *et al.*, 1972; Friis, 1979). However, measurements made in the young of various species post-natally (Calcagno and Rubin, 1963; Gruskin *et al.*, 1970; Horster and Lewy, 1970; Kleinman and Lubbe, 1972) suggest strongly that RPF in *utero* will be low, whether measured in relation to body or kidney weight.

The maturation of GFR, and the rate at which it changes after birth, varies between species. In species such as the guinea pig and human, where nephrogenesis is complete by late gestation (Table 8.12), the post-natal rise in GFR is rapid, and will primarily be due to the rise in systemic arterial pressure and the subsequent perfusion of previously poorly perfused, though apparently structurally mature, nephrons (Merlet-Benichou *et al.*, 1981; Chevalier 1982) and the fall in renal vascular resistance. However, in species such as the rat and rabbit, in which nephrogenesis is not complete at birth, the rate of rise will be slower, since it will include an actual anatomical component in addition to the features mentioned above.

The plasma ultrafiltrate leaves the glomerulus and passes into the proximal convoluted tubule, where, in the adult, approximately 70% of its sodium ions and all of its potassium ions are reabsorbed; there is active reabsorption of glucose and passive reabsorption of non-ionized organic acids and bases and there is secretion by active transport of some organic acids and bases. However, the fetal glomeruli develop before the tubular system and the elongation of the loop of Henle is the last major structural development. Thus, there is a degree of glomerulo tubular imbalance during fetal development (Fetterman *et al.*, 1965), which may persist after birth.

Glucose

The fetal need to conserve its primary energy substrate glucose (Girard *et al.*, 1979) is such that no glucose is present in the urine of either fetal pigs or lambs from at least as early as 0.4 gestation (Parry and Stanier, 1962; D.P. Alexander and Nixon, 1963). This evidence of early active reabsorption is consistent with the presence of alkaline phosphatase in the proximal tubules by the end of the first third of gestation. However, fetal plasma glucose concentrations are low and increasing them by infusion, even to concentrations as relatively low as some 6.5 mmol/l1 results in urinary glucose loss in the fetal lamb (D.P. Alexander and Nixon, 1963). Fetal piglets, near term, conserve glucose very efficiently with a fractional excretion of glucose of only 0.4% (Alt *et al.*, 1984). Adult guinea pigs usually have a fractional excretion of glucose of approximately 1.2% (Merlet-Benichou *et al.*, 1981), but at about 0.71 of the gestational duration the fractional excretion is about 8%, rising to 12% at 0.81 gestation, the period of maximal glomerulo tubular imbalance in this species, and falling thereafter to 2.5% at term (Merlet-Benichou *et al.*, 1981). The immature human kidney appears to have a low transport maximum (T_M) for glucose, even when expressed in terms of body weight or body

surface area (Tudvad and Vesterdal, 1953). These low T_Ms may reflect partly the low GFR and partly the tubular functional immaturity. The low T_M of PAH at this time has already been mentioned above in relation to measurement of renal plasma flow. Fructose is less well reabsorbed than glucose and is present in high concentrations in sheep allantoic fluid from as early as 0.12 gestation, when the mesonephros begins to function (Davies, 1951). This has led to speculation that the secretion of fluid into the largely impermeable allantois helps to keep the uterine cavity expanded (D.P. Alexander *et al.*, 1958) and the membrane layers apposed.

Sodium

Sodium accumulation is a primary requirement for fetal growth. The ion freely crosses the placenta, and the kidney does not therefore 'need' to conserve sodium until term. However, immediately after birth that need becomes paramount, especially since milk has a low sodium concentration and the ability to conserve sodium is in fact well developed in species mature at birth (Spitzer, 1982). It differs from adult salt conservation in that the distal tubule probably plays a more important role *in utero* and in the immediate perinatal period in species which are mature at birth.

Fractional sodium excretion (FE_{Na}) falls progressively during fetal life. In the sheep, it is 40% halfway through gestation, falling to 10% at close to term (D.P. Alexander and Nixon, 1961), paralleled by a marked fall in urinary sodium concentration (D.P. Alexander and Nixon, 1961; Lingwood *et al.*, 1978a). This increase in sodium reabsorption is less than the rise in GFR until late gestation, but exceeds it thereafter (Robillard *et al.*, 1977). Acute experiments in guinea pigs undergoing a saline diuresis showed FE_{Na} to be 27% at 0.7 gestation, and actually rising to 45% at 0.78 gestation (Merlet-Benichou *et al.*, 1981). By term, FE_{Na} was still 20% but by days 2–5 post partum it had fallen to 1%. Human babies delivered early in the third trimester have FE_{Na} of some 5–6% (Siegel, 1982) and can rapidly develop hyponatraemia if not given sodium supplements (Al-Dahhan *et al.*, 1983a). Fetal pigs near term have FE_{Na} of some 2%, falling rapidly to 0.07% by the end of the first week of post-natal life (Alt *et al.*, 1984).

It appears that the progressive rise in GFR with fetal age is accompanied by a parallel rise in sodium and fluid reabsorption across the proximal tubule, as is seen post-natally (Horster and Valtin, 1971; Spitzer and Brandis, 1974). The balance between hydrostatic and colloid osmotic pressures across the peritubular capillaries will change in late gestation, as the colloid osmotic pressure rises (Baum *et al.*, 1971), favouring fluid reabsorption. The main difference in sodium reabsorption in the fetal kidney is that the very short loops of Henle in the fetal kidney do not permit adult-type sodium reabsorption, so that a much greater filtered sodium load is presented to the distal tubule (Aperia and Elinder, 1981). However, considerably more sodium is reabsorbed in this segment than in the adult kidney, partly compensating for the inadequate loops of Henle. It seems highly probable that the raised plasma aldosterone concentrations, characteristic of the neonate (Beitins *et al.*, 1972; Katz *et al.*, 1974; Siegel and Fisher, 1980), may be reflecting increased secretion before birth. The demonstration of inverse associations between urinary sodium–potassium ratio and either plasma concentrations or urinary excretion of aldosterone in the human newborn (Raux-Eurin *et al.*, 1977; Sulyok *et al.*, 1979) and the fetal lamb (Lingwood *et al.*, 1978a; Robillard *et al.*, 1980) strongly suggests a functional interaction between the two parameters in the perinatal period. There is also greater binding of radiolabelled aldosterone in fetal than adult guinea pig kidney (Pasqualini *et al.*, 1972).

Sodium–potassium ATPase (Na^+,K^+-

ATPase) is a well-defined enzyme which drives the sodium pump. Its activity is less in kidney tissue from immature than adult rabbits (Schmidt and Horster, 1977) and rats (Dobrovic-Jenik *et al.*, 1984). The administration of aldosterone to immature but not adult rats was associated with a marked increase in tubular cellular content of Na^+,K^+-ATPase (Aperia *et al.*, 1981), again suggesting aldosterone responsiveness at this age.

Data from human infants delivered early in the third trimester suggest that, although their marked sodium wasting can stimulate activity of the renin–angiotensin system (Stephenson *et al.*, 1991), their adrenal cortices may be unable to synthesize sufficient aldosterone to counter the salt loss at this stage (Sulyok *et al.*, 1979). Similar data are available for fetal lambs, in which a progressively greater aldosterone response to evoked rises in plasma renin activity (PRA) was seen during the last third of gestation. Younger fetal lambs showed no increase in PRA, and hence aldosterone concentration, in response to frusemide-induced sodium loss (Siegel and Fisher, 1980). The prematurely delivered foal continues to lose sodium on challenge with frusemide, despite very large increases in plasma renin and aldosterone concentrations, which may suggest actual unresponsiveness of the tubular cells (Broughton Pipkin *et al.*, 1984).

Potassium

The fractional excretion of potassium is approximately 20% in the middle third of gestation in the lamb, rising to 45% in the last third and 92% by term, falling rapidly thereafter (D.P. Alexander and Nixon, 1961; Robillard *et al.*, 1980). The concentration of potassium in fetal urine, allantoic and amniotic fluid rises steadily towards term in the fetal lamb (D.P. Alexander and Nixon, 1961; Mellor and Slater, 1971; Lingwood *et al.*, 1978b; Robillard *et al.*, 1980) and there is evidence for the tubular secretion of potassium near term in both sheep (Robillard *et al.*, 1980) and guinea pig (Merlet-Benichou and de Rouffignac, 1977). The increased kaliuresis in late gestation could be a function of the increasing GFR, but is more likely to relate to the effects of the increasing plasma concentrations of steroids, especially aldosterone (Robillard *et al.*, 1980), as gestation proceeds.

Water excretion

McCance and Young (1941) observed that the urine of newborn infants was invariably hypotonic to plasma, sometimes very much so. This observation has been made repeatedly, in all animal species studied (e.g. rabbit, McCance and Stanier, 1960; pig, McCance and Stanier, 1960; sheep, D.P. Alexander and Nixon, 1961; Matson *et al.*, 1981; guinea pig, Boyland *et al.*, 1958; Chez *et al.*, 1964). Indeed, the osmolality of fetal pig urine collected from the bladder was only 13% of plasma osmolality in mid-gestation, reflecting the extremely low urinary urea at this time (McCance and Stanier, 1960).

Since transplacental water flux is substantially higher than that needed for normal fetal growth (Flexner and Gellhorn, 1942), the production of a hypotonic urine is not surprising. The greater part of the glomerular filtrate is reabsorbed in the proximal tubule, where fluid transport is isosmotic. The passive reabsorption of water following active sodium transport is determined by the water permeability of the proximal tubule, which is increased in the immature kidney (Horster and Larsson, 1976), and the oncotic and hydrostatic pressure gradients in the lumen, interstitium and peritubular capillaries (see above). The remaining water reabsorption occurs mainly in the countercurrent multiplication system of the loop of Henle (anatomically immature *in utero*; see above) and the concentrating tubules.

The two main factors militating against efficient concentration, once fluid has left the proximal tubule, are the very low medullary

sodium and urea concentration and a relative insensitivity of the duct to vasopressin, possibly mediated by the low cortisol concentrations *in utero* (Towstoless *et al.*, 1989). Stanier (1972) studied the development of intrarenal solute gradients and described marked interspecies variability. For example, the sodium concentration gradient between cortex and papilla in the fetal pig kidney is very small indeed up to term, and only steepens post-natally, while in fetal lambs, by late gestation, the sodium concentration gradient is steep. The urea gradient is also very small in fetal pigs, and because of their extremely fast rate of post-natal growth only builds up relatively slowly thereafter. McCance and Widdowson (1957) suggested that this was likely to be a characteristic of species which grow very rapidly **post partum**, but the pig is also anomalous in having a water requirement for urea excretion.

Common to all mammalian fetuses, is the fact of earlier development of inner cortical nephrons. These take the largest proportion of the renal blood flow and, since their efferent arterioles supply the medullary vasa recta, there will also be a 'washout' effect, impeding the build-up of a solute gradient.

Although the urine is characteristically hypotonic at birth, the term fetus can concentrate it to approximately 150–200% plasma osmolality (Robillard and Weitzman, 1980), although this is markedly less than the adult's concentrating ability. The chronically catheterized fetal lamb appears to have functional volume and osmoreceptor controls for arginine vasopressin (AVP) secretion (Ross, 1988) and atrial natriuretic peptide secretion (Ross *et al.*, 1987), and shows a gradual increase in free water reabsorption in late gestation (Robillard *et al.*, 1979). The fetus can respond to increases in maternal plasma osmolality by increasing its urinary osmolality (Lumbers and Stevens, 1983) and markedly decreasing the urinary flow rate. The fetus will also respond appropriately to the infusion of exogenous AVP in the last quarter of gestation, by decreasing free water clearance and increasing urinary osmolality (Lingwood *et al.*, 1978b; Robillard and Weitzman, 1980). It seems likely, however, that the fetal kidney is less sensitive to AVP than is the adult, and Joppich *et al.* (1981) suggested that this might be due to inhibitory effects of the high intrarenal concentrations of prostaglandin E_2 at this time. It might also be related to a lesser response of adenyl cyclase to AVP (Schlondorff *et al.*, 1978).

Protein

The urine from the human fetus in the middle trimester contained no or trace amounts of protein when tested with trichloroacetic acid (Vernier and Birch-Andersen, 1962) but more sophisticated detection techniques have shown that urinary proteins of various kinds are present in human urine from at least mid-gestation (Kronquist *et al.*, 1984). These included albumin, α–fetoprotein, transferrin and Tamm–Horsfall protein. However, in the last quarter of gestation the relative abundance of serum-type proteins in the urine was much less and resembled the adult pattern. Whereas urine from mid-gestation contained a complex assortment of polypeptides of molecular weight 12 000–40 000 daltons, the mechanisms increasing the reabsorption of small proteins appeared to have matured. The greater permeability of younger fetal glomerular capillaries (Vernier and Birch-Anderson, 1962) presumably accounts for the relatively large early concentrations of urinary protein. The appearance of Tamm–Horsfall protein in the urine of the fetal rat parallels functional development of the thick ascending limb of the loop of Henle, and may be related to the development of impermeability to water in this segment.

The anatomical and functional development of the fetal kidneys, in which ontogeny so well recapitulates phylogeny, is among the most complex aspects of fetal physiology. The marked effects of anaesthesia and sur-

gery on renal function also hinder its study, and areas such as the renal effects on acid–base balance *in utero* remain poorly understood.

8.3.5 THE REPRODUCTIVE SYSTEM

Like the developing kidney, with which it is closely associated, the fetal reproductive system needs to grow and develop, but will not have functional demands made on it until after birth.

(a) Structure

The primitive gonads develop from the genital ridges which form on the mesonephroi. They consist of coelomic epithelium, an underlying mesenchyme of mesonephric origin, and the germ cells. The germ cells arise in the yolk sac very early in gestation and migrate to the germinal ridges by amoeboid movement, multiplying as they go. The germ cells and coelomic epithelium together form the cortical germinal epithelium (Figure 8.18), while the inner mesenchyme and the mesonephric tubules form the medulla.

Differentiation in the chromosomal male fetus precedes that in the female, as the primary cords grow, branch and attract germ cells, becoming the medullary spermatogonia. The cortex thins and the tunica albuginea forms (Figure 8.18). In the chromosomal female fetus, the cortex continues to grow, with the germ cells embedded in it developing as oocytes.

There is sexual differentiation in the timing of the onset of meiosis in germ cells, which occurs substantially earlier in the female than in the male fetus. Meiosis of oogonia in the human female fetus begins at around 8 weeks' gestation, resulting in haploid primary oocytes, arrested in prophase and remaining like this until individual oocytes start to mature further at puberty. There is no further mitotic division, and the number of primary oocytes thus falls thereafter. In the male, however, mitotic division of the spermatogonia continues, and meiosis is only initiated at puberty, continuing throughout life. It is interesting to note that the mesonephros may secrete a 'meiosis-inhibiting hormone'. In those species, such as the pig and rabbit, in which mesonephric function continues until relatively late in gestation

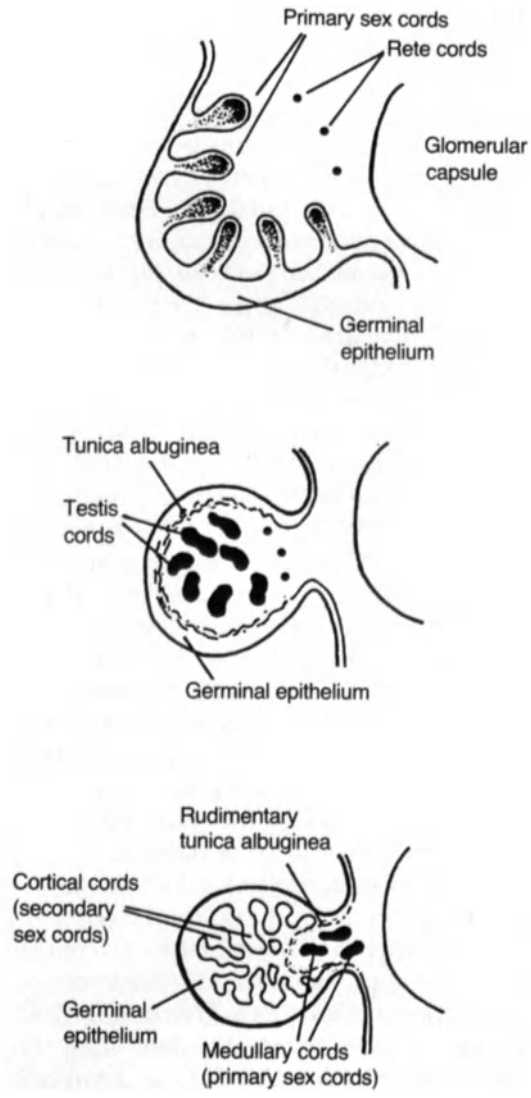

Figure 8.18 General gonadal development in the mammalian fetus.

Table 8.13 The proportion of gestation at which gonadal differentiation in the chromosomal male fetus develops varies widely between species. Data from: Attal, 1969; Black *et al.*, 1969; Catt *et al.*, 1975; Tanner, 1978; MacDonald, 1979; Pointis and Latreille, 1986.

Species	*Proportion of gestation*
Man	**0.23**
Sheep	0.24
Pig	0.25
Guinea pig	0.35
Rabbit	0.55
Rat	0.82

(Table 8.13), the onset of meiosis in the oogonia occurs after sexual differentiation, whereas in those species having small and minimally functional mesonephroi, such as the rat and human, the onset of meiosis and sexual differentiation is approximately synchronous (Grinsted and Aagesen, 1984).

Gonadal differentiation is the first sexual differentiation to occur and is initiated through the presence of a Y chromosome. The testis determining factor (TDF), a 120 kilobase segment of the short arm of the Y chromosome, induces testicular differentiation by the fifth or sixth week of pregnancy in the human fetus (N. Barnes, 1992). The presence of testes thus indicates the presence of a Y chromosome (unless there is a translocation of the TDF onto the paternal X chromosome, in which case an XX male or XX true hermaphrodite can develop). Male sex is also associated with the male histocompatibility antigen, the H-Y antigen, a cell-surface protein which is found in the males of all mammalian species. The function of this protein, and the nature of its relationship to TDF, is not clear. Subsequent male differentiation requires testicular hormone production from the Leydig cells and Mullerian inhibitory factor (MIF) from the Sertoli cells. Testosterone stimulates the Wolffian ducts to become the epididymis, vasa deferentia and seminal vesicles. MIF causes local regression of the Mullerian ducts. Androgenic stimulation is also necessary for differentiation of the external genitalia which, without such stimulation, are female in character regardless of chromosomal and gonadal sex.

The Mullerian ducts are retained in the female fetus, and form the Fallopian tubes, uterus and upper vagina; it is the Wolffian ducts which regress in this sex, although contributing to the ureter and the trigone of the bladder. In both sexes the external genitalia develop from the urogenital sinus and tubercle, and are the last part of the reproductive tract to differentiate (see this series, Volume 2, Chapter 4).

Male development is, therefore, an active embryological process which is complete by 13 weeks of the human pregnancy. In contrast, female development may be described as the 'default option', a passive process which proceeds even in a castrated fetus irrespective of the genetic sex (Jost, 1953).

The importance of hormonal change in governing development and function of the reproductive system cannot be overstated, and is the subject of the rest of this section.

(b) Hormonal effects

Testosterone

The onset of testosterone secretion by the fetal testis appears to be governed by placentally produced gonadotrophin (Huhtaniemi *et al.*, 1977). It is this locally produced testosterone which is responsible for the male development of the testis. The fetus is provided with a rich source of steroid precursors such as progesterone and pregnenolone from the placenta, and does not, therefore, need the capacity to take acetate or cholesterol through the biosynthetic chain to testosterone. However, there are species differences in substrate preference, in that both newborn mouse and fetal human testes can utilize either progesterone or pregnenolone equally *in vitro*, while fetal rabbit testis preferentially

utilizes pregnenolone in the synthesis of testosterone (Siiteri and Wilson, 1974). Probably very little maternal testosterone crosses the placenta (Rigaudière and Després, 1986).

The development of a synthetic capacity for testosterone either precedes (rabbit and rat, Black and Christensen, 1969; sheep, Attal, 1969) or appears to be simultaneous with (guinea pig, Black and Christensen, 1969; human, Siiteri and Wilson, 1974) the time of testicular cytodifferentiation and enlargement of the interstitial cells. These interstitial cells contain substantial quantities of tubular smooth endoplasmic reticulum and are the primary source of testicular androgens (Black and Christensen, 1969; Veyssiere *et al.*, 1977).

The concentration of testosterone in the testis reaches a peak during the differentiation of the internal genital tract, and falls thereafter (rabbits, 0.63 gestation, Catt *et al.*, 1975; Veyssiere *et al.*, 1976; man, 0.36 gestation, Siiteri and Wilson, 1974; pig, 0.31 gestation, A.A. MacDonald, 1979). The decrease in testosterone formation coincides in man with a regression in both the size and number of the interstitial cells during the second half of gestation (Siiteri and Wilson, 1974) and is associated with an accumulation of 17α-hydroxypregnenolone, implying a change in enzyme activity. Circulating testosterone concentrations are also high during internal genital differentiation in fetal pigs, cattle and humans, falling thereafter (Veyssiere *et al.*, 1976). However, although circulating testosterone concentrations rise sharply after birth in some species (e.g. rabbit, Veyssiere *et al.*, 1976; pig, Ponzilius *et al.*, 1986), in others plasma testosterone continues to fall for some time post-natally, with only a delayed and transient rise (e.g. human, Tanner, 1978; rat, Huhtaniemi *et al.*, 1982).

The fetal ovary appears to have little capacity to synthesize testosterone, but such as there is peaks at much the same time as the testicular synthetic capacity. In the rabbit, fetal ovarian testosterone concentrations are only 2% of testicular values at the peak (0.65 gestation, Veyssiere *et al.*, 1976). Plasma testosterone concentrations in newborn female rabbits, pigs and rhesus monkeys are also lower than in males at birth (Veyssiere *et al.*, 1976; Huhtaniemi, 1977; A.A. MacDonald, 1979), and in this they differ from such species as cattle, sheep and man, in which there are no sex-linked differences in plasma testosterone at birth.

While locally-synthesized testosterone is the active inducer of testicular and Wolffian duct differentiation, the embryonic anlagen of the external genitalia and the prostate primarily synthesize dihydrotestosterone (2-OH-testosterone). In man, the 2-OH-testosterone production per milligram of tissue in the urogenital sinus is already high by 0.2–0.25 of gestation, considerably before differentiation of this region. The Mullerian and Wolffian ducts show no *in vitro* capacity to synthesize 2-OH-testosterone before the end of the first trimester, while none is evident in the epididymis until about halfway through gestation (Siiteri and Wilson, 1974).

Testosterone plays another important role in the development of the reproductive system. The testis must remain as a non-cyclical organ, while the ovary becomes cyclically active. Determination of cyclicity is made through the action of testosterone on the hypothalamus, and once exposure to testosterone has occurred the change is irreversible and 'maleness' is dominant. This 'sensitive period' appears to be at the start of the second trimester in man (G.W. Harris and Levine, 1965), but in species born in a less mature state may not occur until after birth. This is so, for example, in the rat, in which the 'sensitive period' is around 5 days post-natally (G.W. Harris and Levine, 1965). Such differences may account for the sexual dimorphism of gonadotrophin release seen in the fetus of some species but not others.

Testosterone must be converted either to oestradiol or to 2-OH-testosterone in the

hypothalamus before it can exert its effect on the brain. Binding sites for both hormones are present in the guinea pig brain halfway through gestation, and, like man, this is a species in which hypothalamic dimorphism is of prenatal onset (Pasqualini *et al.*, 1977; Rigaudière and Després, 1986). Such sites are not present in the rat brain until after birth (Pasqualini *et al.*, 1977) when hypothalamic oestradiol concentrations are high in male pups (Rhoda *et al.*, 1987). Once sexual differentiation of the hypothalamus has occurred, growth rates are enhanced in the male fetus.

Oestrogens

The gonadal capacity to synthesize oestrogens lags behind that for testosterone. Rat fetal testicular cells have a very low capacity for aromatization, and undetectable oestrogen production (Tsai-Morris *et al.*, 1986). In the sheep, no gonadal oestrogens are detectable before halfway through gestation (Attal, 1969). However, measurable plasma concentrations of sulphated oestrogens are found in fetal guinea pigs (Pasqualini *et al.*, 1977), mostly unbound. This may be peculiar to the guinea pig which has either very low or absent concentrations of α-fetoprotein (AFP), which in other species binds the oestrogens. Fetal guinea pigs also have high-affinity oestradiol binding sites in both uterus and testis, from at least halfway through gestation, rising towards term (Sumida and Pasqualini, 1979), although those in the testis are only present at one-seventh the concentration of those in the uterus (Pasqualini *et al.*, 1976). Conversely, fetal rat gonads have very low concentrations of oestradiol receptors (Tsai-Morris *et al.*, 1986).

Gonadotrophins

Although it is placental, rather than pituitary, gonadotrophin which initially stimulates testosterone production by the future testis, the fetus in later gestation does acquire a functional hypothalamo-pituitary axis quite independent of the mother's. The degree to which this influences the fetal gonad depends largely on the general maturity of the fetus at birth.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are present in the anterior pituitary gland of pigs, cattle and man from about one third of gestation (Kiser *et al.*, 1975; J.A. Clements *et al.*, 1976; Ponzilius *et al.*, 1986). The peak pituitary concentrations occur before birth in both sexes, and appear not to differ with gender. The concentrations fall towards term. Gonadotrophin-releasing hormone (GnRH) may be present earlier in the hypothalamus (e.g. at 0.16 gestation in man) but *in vivo* appears not to interact with the pituitary until considerably later (see below).

Basal plasma concentrations of LH are measurable soon after the hormone can be detected in the pituitary gland. Release is probably pulsatile, as in the adult, but the number and amplitude of such pulses is low before birth (Ponzilius *et al.*, 1986), falling to a nadir at term. Basal concentrations have been variously reported as higher in the female than the male fetus (rhesus monkeys, Resko and Ellingwood, 1985; humans, J.A. Clements *et al.*, 1976); lower (cattle, Kiser *et al.*, 1975) or the same (pigs, A.A. MacDonald, 1979; Ponzilius *et al.*, 1986). The lack of sexual differences in plasma LH in fetal pigs has been attributed to an apparent lack of negative feedback by the moderate testosterone concentrations of the fetal male piglet (Ponzilius *et al.*, 1986).

By contrast, the basal FSH concentration is substantially higher in human female than male fetus during the second trimester (Gennser *et al.*, 1976), but even so the concentration in males exceeds that in the maternal circulation. FSH in the female fetus rises during the second trimester, falling thereafter to very low concentrations at term (Clements *et al.*, 1976); this fall is not seen in the male fetus.

Both LH and FSH plasma concentrations rise dramatically after birth in humans and pigs, LH concentrations being greater in the human male fetus (Clements *et al.*, 1976; Ponzilius *et al.*, 1986) but the concentrations then fall and remain low until beginning their prepubertal changes.

The concentration of gonadotrophin receptors appears to be determined by endogenous hormone concentrations in the fetus, unlike the adult. Where concentrations are low, as in the rat, treatment with exogenous hormone up-regulates the receptors, and allows biological responsiveness to the hormone to emerge (Huhtaniemi *et al.*, 1982; Tsai Morris *et al.*, 1986). The association constants of the LH-hCG receptors of fetal rabbit testis halfway through gestation are identical to those of adults, suggesting that biological activity of the gonadotrophins will depend more on receptor number (Catt *et al.*, 1975). Binding of labelled hCG by fetal rabbit and rhesus monkey testis rises until late mid-gestation, but is stable thereafter, while ovarian binding sites are present in much lower concentrations (Catt *et al.*, 1975; Huhtaniemi *et al.*, 1977). In a series of precisely timed observations, Catt *et al.* (1975) were unable to show a temporal dissociation between the appearance of the LH-hCG receptor at 0.60 of gestation and the ability of the fetal rabbit testis to synthesize testosterone.

The mutually inter-regulatory role of the gonadotrophins and gonadal steroid production is well documented *in utero*. The crudest demonstration of this is that, following castration of male fetuses, LH and FSH concentrations rise substantially (Resko and Ellingwood, 1985; Ponzilius *et al.*, 1986), a rise which is blocked by the administration of testosterone or 2-OH-testosterone. This rise in gonadotrophin concentration is much smaller in some species than others, presumably relating to immaturity of the pituitary–gonadal axis. In the pig, plasma LH concentrations only rise after birth following antenatal castration; in this respect, as in many others, the pig differs from other species of similar size and gestation length. Conversely, decapitation of the near-term fetal rat is associated with retardation of testicular growth and the volume and number of the Leydig cells (Eguchi and Morikawa, 1968). This is a species which does not have a placental gonadotrophin, so that pituitary–gonadal interaction must occur *in utero* for normal development.

The administration of LH or hCG in late pregnancy stimulates testicular testosterone production in all species studied. This, of course, differs from the effects of similar administration to adults, in which there is a short-lived increase in testosterone secretion, followed by receptor down-regulation and blockade of synthesis. The transition to an adult-type response is well documented in the neonatal rat (Huhtaniemi *et al.*, 1982) and is paralleled by the disappearance of AFP, which binds oestrogen, from the circulation, and the rapid development of oestradiol receptors in the testis, both allowing the onset of negative feedback by oestrogens on testosterone synthesis.

Hypothalamic effects on the gonads are the last to manifest themselves in the hormonal chain of development of the reproductive system. *In vivo*, fetal calves at 0.40 of gestation respond to GnRH by increasing plasma LH, female calves showing a substantially greater response. Serum testosterone also rises following GnRH administration in male, but not female, fetal calves (Kiser *et al.*, 1975). However, GnRH was without effect on human fetal FSH or LH/hCG during the second trimester (Gennser *et al.*, 1976), although fetal pituitaries *in vitro* released gonadotrophins in response to GnRH at this age. Experiments with fetal lambs, rabbits and monkeys *in vivo* near term showed stimulation of LH and testosterone secretion, although high doses of GnRH were needed in the lambs and monkeys (Foster *et al.*, 1972; Huhtaniemi *et al.*, 1977; Veyssiere *et al.*,

1977). This may be due to the low pituitary concentrations of gonadotrophins at this time. The response tended to fall towards term. The fall in basal testicular testosterone production towards term (see above) is, thus, likely to be related to the declining concentrations of gonadotrophins, rather than to immaturity of the hypothalamo-pituitary–gonadal axis *per se*.

Thus, by the time of birth the anatomical and hormonal differentiation necessary for reproductive function is to a considerable extent complete. The fascinating thing is that the system is then largely held in abeyance, until such time as maturation of the other body systems will allow the female to support a pregnancy. How this state of quiescence is maintained remains something of an enigma.

8.3.6 THE LIVER

(a) Structure

The liver develops as a large diverticulum of the foregut, from which endodermal cells detach themselves and surround the developing network of vitelline veins. The hepatic venous circulation is formed by the anastomosis and remodelling of the anterior vitelline and the umbilical veins, while the portal vein is derived from the caudal ends of the vitelline veins. The hepatocytes are surrounded by a network of developing bile canaliculi which appear to bud off from the hepatic plates. The bile ducts and ductules run in parallel with the branches of the developing portal venous and hepatic arterial systems (Kaufman, 1992).

In the fetal lamb, the umbilical vein enters the liver and gives rise directly to portal branches to the left lobe of the liver. The ductus venosus then arises from the umbilical vein, which continues to join the portal vein, the portal branches to the right lobe of the liver arising from near this junction. The communication between umbilical and portal veins is sometimes referred to as the portal sinus, but no true anatomical sinus exists (Rudolph and Rudolph, 1992). Hence, part of the flow from the placenta by-passes the liver via the ductus venosus which joins the inferior vena cava (IVC) in a joint connection with the left hepatic vein. The right hepatic vein joins the IVC through an adjacent, but separate, orifice. In both the sheep and the primate fetus, the proportion of umbilical venous return crossing the ductus venosus, and avoiding the hepatic microcirculation, averages slightly more than half (Edelstone and Rudolph, 1979). Within the IVC, this well-oxygenated blood from the placenta, which returns via the ductus venosus, does not completely mix with either the poorly oxygenated blood returning from the lower body or the hepatic venous blood. The stream of highly oxygenated blood preferentially crosses the foramen ovale and contributes to the cerebral arterial supply (Rudolph, 1983), this preferential streaming being most pronounced in the fetal primate.

In the horse, however, the caudal end of the ductus venosus is obliterated in early gestation (e.g. R.J. Barnes *et al.*, 1979). Thus, in this species the umbilical venous blood actually perfuses liver tissue before draining into the inferior vena cava via the hepatic veins. It used to be thought that a ductus venosus was also absent in the fetal pig, but later work (R.J. Barnes *et al.*, 1979) has confirmed the presence of a functional hepatic by-pass in late gestation, even though its anatomical origin may differ from that in other mammals.

About three-quarters of total hepatic blood flow *in utero*, in the sheep and primate, is derived from the well-oxygenated blood (saturation 80%) returning from the placenta (Rudolph and Rudolph, 1992). The remainder is supplied by poorly oxygenated portal venous blood, there being minimal supply from the hepatic artery *in utero*. The left and right sides of the liver receive different blood supplies in fetal life. The left lobe of the liver is perfused with relatively well-oxygenated

blood from branches arising directly from the umbilical vein. The blood perfusing the right side of the liver is poorly oxygenated (Dawes, 1968), with an oxygen saturation little higher than that of the portal venous blood (27%) in the newborn lamb. The higher proportion of haemopoietic foci in this area (Emery, 1956) presumably reflects this, erythropoiesis being stimulated by hypoxia (section 8.3.2, 'Blood and oxygen transport').

(b) Function

Bile production

Experimental study of fetal bile production is technically difficult because the bile canaliculus is relatively inaccessible and therefore less is known about the ontogeny of this process than many other aspects of fetal liver function. In the fetus, nutrition is via the placenta, not by the gastrointestinal tract, so that the mechanisms for the release of bile, including cholecystokinin release, are not invoked, and the need for detergent and micellar-forming properties of bile in the intestine is absent. Perhaps not surprisingly then, there is a lower rate of spontaneous bile flow in the fetal sheep near term than in the adult sheep (Hardy, 1980). Bile can also be secreted from the gall bladder from around halfway through gestation in man, and there appears to be some enterohepatic circulation (see Grand *et al.*, 1976). The fetal intestine is bacteriologically sterile, and it must therefore be assumed that free deoxycholic acid, which is formed by bacterial 7-α dehydration of cholic acid, does not occur *in utero* (Gustafsson *et al.*, 1985). However, the primary bile acids are found in meconium, which suggests that some hydroxylation of cholic acid can occur. Human fetal liver *in vitro* can also hydroxylate taurodeoxycholate at least during the second trimester, which adult liver only does minimally (Gustafsson *et al.*, 1985). This presumably provides an alternative pathway preventing the build-up of the toxic conjugated deoxycholic acid.

Bilirubin metabolism

The fetal liver plays a major role as a haemopoietic organ; indeed, in early fetal life haemopoietic cells outnumber hepatocytes and contribute to the proportionally greater size of fetal than adult liver. This aspect of fetal liver function is discussed in section 8.3.2, 'Blood and oxygen transport'. Bilirubin is one of the major endpoints of red cell breakdown. In the adult, free bilirubin is rapidly bound by plasma albumin. Almost all of the bilirubin which is absorbed into the hepatic cells is then rapidly conjugated, mostly to glucuronic acid but a little to the sulphate and other substances, before being excreted into the bile canaliculi. However, in the fetus, although red cell destruction proceeds and bilirubin is produced, plasma albumin is relatively low (e.g. Baum *et al.*, 1971; Spitzer and Edelmann, 1971; Cartlidge and Rutter, 1986) and the ability to conjugate is diminished due to the absence or very low concentrations of hepatic glucuronyl transferase. In the rat, for example, there is almost no conjugation before 0.82 gestation and the free bilirubin concentration is two to three times higher than in the adult, partly due to slower placental transport in this species than in the guinea pig or primates (Muraca *et al.*, 1986). The ability to monoconjugate precedes deconjugation, and by term only 6% of the bilirubin in fetal rat plasma is conjugated. However, there is a rapid rise in conjugating ability after birth, and within 24 h approximately 40% of plasma bilirubin is conjugated in this species (Muraca *et al.*, 1986). The process takes longer in other species, such as man and horse (e.g. Gartner, 1981; J.W. Harvey *et al.*, 1984). Hence, there is a period of neonatal hyperbilirubinaemia (neonatal jaundice) due to the breakdown of fetal red cells with increased production of bilirubin, enhanced bilirubin reabsorption from the intestine via the enter-

ohepatic circulation and a slowly improving ability to conjugate bilirubin. It should, however, be remembered that placental transfer of bilirubin, the main excretory route before birth, depends on the bilirubin remaining unconjugated, as a relatively lipid-soluble polar substance. 'Inadequate' conjugation may thus be an adaptive, rather than an immature, mechanism. Bilirubin glucuronides have been identified in the amniotic fluid of pregnancies complicated by fetal haemolysis due to blood group incompatibility (Rosenthal, 1992). This suggests that the fetal capacity to conjugate bilirubin may be stimulated by raised circulating bilirubin levels, a condition which does not normally obtain until after birth because of the efficiency of the placenta. The lower conjugating ability of the fetal liver means that the ability of the fetus and newborn to metabolize and excrete drugs is impaired by comparison with the adult.

Plasma protein production

The liver makes other contributions to the composition of the blood (Dancis *et al.*, 1957). Fetal hepatocytes synthesize α-fetoprotein. This occurs from 0.15 gestation in man, and plasma levels reach a peak halfway through gestation, by which time all hepatocytes are synthesizing the protein. Concentrations then fall as the cells are switched progressively to the production of albumin (Lindblad, 1976; Stone, 1981). Even so, plasma albumin concentrations tend to be low at birth, especially after premature delivery (Spitzer and Edelmann, 1971; Baum and Harris, 1972; Cartlidge and Rutter, 1986). The function of α-fetoprotein is unknown, but it may serve as a primitive form of albumin or have an immunoregulatory function (Ruoslahti and Hirai, 1976). Very elevated concentrations at term are seen in infants born with a teratoma. α-fetoprotein also acts as one of the 'tumour markers' if plasma concentrations increase in later life, being particularly associated with hepatomas and seminomas.

The fetal liver also synthesizes various coagulation factors, particularly prothrombin and factors VII, IX and X. This synthesis is particularly susceptible to vitamin K deficiency, which is therefore associated with haemorrhagic disease of the newborn (McNinch and Tripp, 1991). These clotting factors are present in lower concentrations in late fetal and early neonatal life than in adulthood in all species studied (Dancis *et al.*, 1957).

Glycogen storage

At the moment of birth, the fetus is removed from its continuous and regulated supply of glucose from the mother, and must rapidly switch to utilizing the high-fat relatively low-carbohydrate diet of mother's milk. There is usually an interim period before the newborn begins to suckle, and during this phase the hepatic glycogen stores play a vital role in maintaining normoglycaemia. These are built up during gestation in all species studied (Figure 8.19) at rates which are partly dependent on length of gestation, species with longer gestations starting to lay down stores relatively early, and in a more leisurely fashion, than those with shorter gestations (Shelley, 1961; Randall and L'Ecuyer, 1976).

By term, hepatic glycogen concentrations are twice those in the adult (approximately 12 mg per g wet weight compared with 6 mg per g wet weight; Lafeber, 1981). Glycogen synthesis *in utero* is under hormonal control, with the hypothalamus playing an integrative role (see Girard, 1986). As well as insulin, the insulin-like factor and cortisol, epidermal growth factor (EGF) appears to be involved in glycogen deposition in the fetal liver (Freemark, 1986). The concentration of EGF binding sites rises rapidly from 0.85 gestation to term in fetal mouse liver, in parallel with the ability to stimulate the incorporation of labelled glucose into glycogen and the rise in

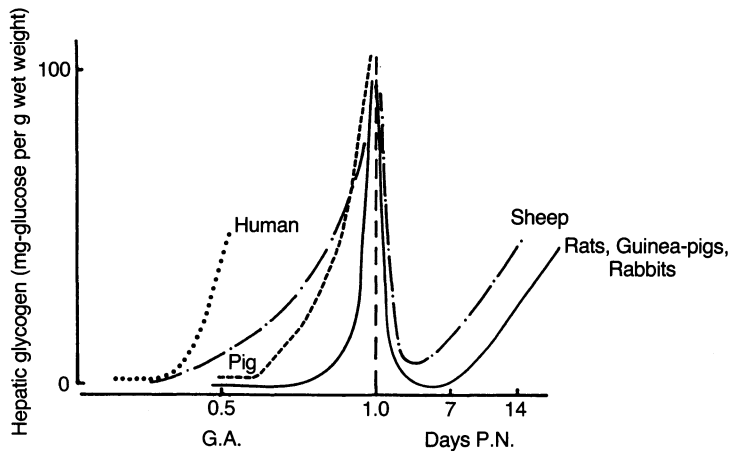

Figure 8.19 There is a marked rise in hepatic glycogen concentration towards term in all species. The transition to an extrauterine environment and activation of the digestive system is accompanied by an abrupt fall in these stores, which can then take several weeks to reach adult values. (Redrawn from Shelley, 1961, and Randall and Ecuyer, 1976). GA, gestational age; PN, post-natal.

hepatic glycogen deposition. A stimulatory effect of EGF on the size of fetal lamb liver has also been reported (Thorburn *et al.*, 1981), but this appears to be a generalized phenomenon, rather than specifically related to glycogen deposition.

It is worth remembering that, although the fetal liver markedly accumulates glycogen as term approaches, other tissues are also storing this energy substrate. Thus, in species which have very little fat at birth, such as piglets, the skeletal muscle accumulates substantial glycogen stores before birth, some 90% of its energy reserves being in this form (Randall and L'Ecuyer, 1976). Conversely, term fetal rabbits have low glycogen concentrations in their skeletal muscle (see Dawes, 1968). Instead they have considerable brown and white adipose tissue, providing fuel sources for heat production and gluconeogenesis which can be switched on after birth. For example, beta-oxidation of fatty acids by rabbit hepatocytes is eightfold greater in 24-h-old newborn rabbits than in fetal rabbits (Duee *et al.*, 1985). Hepatic fatty acid oxidation also increases in the rat during the suckling period, compared with fetal life, and falls to adult levels during the weaning period (E. Bailey and Lockwood, 1973; Zimmermann *et al.*, 1986). Such differences may also relate, in part, to the fact that the piglet is active immediately after birth, and needs a direct energy supply for running about, while the newborn rabbit lives in a nest and has minimal requirements for skeletal muscular activity.

Gluconeogenesis

The process of birth and the interruption of a continuous food supply very rapidly deplete the hepatic glycogen stores (Figure 8.19; Shelley, 1961). The enzymes necessary for gluconeogenesis are present in the fetal liver of most species except the rat, development again occurring relatively sooner in those species with longer gestation periods. However, providing that the mother is receiving an adequate nutrient intake, intrauterine gluconeogenesis does not seem to occur in species as diverse as the rat, the sheep and man. Should the mother rat, rabbit, guinea pig or sheep be fasted, hepatic enzyme induction can occur *in utero*, espe-

cially that of phosphoenolpyruvate carboxykinase (PEPCK), essential in the early steps of gluconeogenesis which can then be initiated. Birth itself is an important stimulus to cytosolic PEPCK induction. Glycerol is another substrate which can be utilized for fetal gluconeogenesis (M. Gilbert, 1977) and the enzymes concerned in its metabolism, primarily glycerol kinase and glycerol-3-phosphate (G3P) dehydrogenase, are present in fetal liver (Vernon and Walker, 1970; Sadava *et al.*, 1987). Hepatic mitochondrial G3P dehydrogenase rises sharply after birth, which may suggest the activation of a glycerol phosphate shuttle. The whole topic of perinatal gluconeogenesis has been reviewed by Girard (1986).

Amino acid breakdown

The degradation of amino acids occurs mainly in the liver, and has been studied in the fetus largely in terms of induction of the various enzyme systems. It is, however, important to remember that the fetus's primary need is to synthesize protein, rather than to break it down. The fetal lamb can oxidize amino acids (F.C. Battaglia and Meschia, 1988) and can therefore use them as an alternative energy source, as can the guinea pig (Lafeber, 1981). The rat, however, appears unable to do this (Snell and Walker, 1973). Many liver enzymes are present but with low activity, possibly explained by the low blood flow and high insulin concentrations pertaining *in utero*. The free amino acid concentration, with the exception of valine and cystine, is high in fetal liver, following placental transfer, and this might enhance the enzyme activities needed for nucleic acid synthesis. However, it has been suggested that various hepatic enzymes such as tyrosine aminotransferase are inhibited *in utero* (Raiha, 1971) and that the increase in PaO_2 or hepatic blood flow post-natally allows full activity of the enzyme. Tryptophan oxygenase also increases markedly immediately after birth (Lindblad, 1976). A summary of amino acid metabolism during fetal life is available to the interested reader (Lindblad, 1976).

Fatty acids

Fatty acids are mostly used in early gestation for membrane formation (Naughton, 1981), and as triglycerides for fat storage in later gestation. The guinea pig, relatively mature at birth, has a peaking of the enzymes concerned with fatty acid synthesis and of such synthesis at two-thirds of the way through gestation, coinciding with the development of the smooth endoplasmic reticulum and with a peak of the enzymes involved in NADPH production (Lafeber, 1981). The exception is acetyl CoA carboxylase, which is barely detectable in the guinea pig liver at term (C.T. Jones and Ashton, 1976). In species less mature at birth, such developments occur in the immediate perinatal period.

Urea synthesis

Hepatic cyclic urea synthesis is a complex process involving both mitochondrial and cytoplasmic enzymes. The need for urea synthesis *in utero* is small since protein synthesis exceeds degradation at this age. It is possible that the primary role of the urea cycle antenatally is to synthesize arginine, since relatively little crosses the placenta. Arginine synthetase is, however, still relatively low at birth (Raiha and Suihkonen, 1968a). Increasing synthetic capacity for urea can be demonstrated *in vitro* from mid-gestation in man, and in the last third of gestation in the guinea pig and rhesus monkey, in parallel with the development of ornithine transcarbamylase (Raiha and Suihkonen, 1968a; Lafeber, 1981; Holzgreve *et al.*, 1985). In species of short gestation, such as the rat, the capacity for cyclic urea synthesis *in utero* is very low, but rises rapidly in the first 24 h post-natally to

Table 8.14 Some markers of the anatomical development of the stomach in various mammalian species in relation to the proportion of gestation at which they are identified. Data from: Hill, 1956; Hayward, 1967; Helander, 1969; Arey, 1974; de Lemos, 1977; MacDonald *et al.*, 1982.

Species	*Gastric pits*	*Mucous neck cells*	*Chief cells*	*Intestinal villi*
Man	**0.15**	**0.15**	**0.30**	**0.15**
Sheep (abomasum)	0.46	?	0.58	0.27
Pig	0.63	?	?	?
Rabbit	0.77	0.97	0.77	?
Rat	0.86	0.86	1.27	?

some 25% of adult capacity (Kadowaki *et al.*, 1983). This rise in capacity parallels the development of argininosuccinate synthetase activity, which seems to be rate-limiting in this species (Raiha and Suihkonen, 1968b).

8.3.7 THE GASTROINTESTINAL TRACT

The gastrointestinal (GI) tract is one of the organ systems which contributes little to fetal well-being before birth, but which becomes immediately vital in the post-natal period. Maturity of the GI tract varies widely at birth. Thus, in the guinea pig the anatomical structure and hormone and enzyme systems of stomach and small intestine are effectively mature at birth. In the lamb, on the other hand, the anatomical and histological development of the forestomach (rumen, reticulum and omasum) lags markedly behind that of the abomasum until birth, with rapid catch-up growth thereafter (Nickel *et al.*, 1973); the abomasum is, however, sufficiently developed to allow the efficient digestion of colostrum and milk. Species born after a very short gestation, such as rats, have functionally very immature GI tracts at birth, and the direct intestinal transfer of macromolecules continues for some 3 weeks post-natally (see below). The phased development of gastric enzyme systems in man has clinical relevance in relation to the selection of appropriate feeding for the very prematurely delivered human infant, since survival from before the end of the second trimester is now possible in specialized units. This topic is not considered further in this chapter on normal fetal physiology, but is mentioned as an example of the numerous ways in which basic and applied research are directly related in this area.

(a) Structure

Although the stage of gestation at which gastric development is initiated varies between species its early stages are similar in all species, with developmental maturity progressing from the proximal to the distal end of the tract. Once the dilatation in the primitive foregut which will form the stomach has arrived at its definitive position, the columnar cells of the gastric mucosa are pushed progressively into folds with mesodermal 'cores', forming primitive villi. Gastric pits form at the base of these folds, the precursors of the gastric glands. The rate of development of these glands is proportionally slower in species with shorter gestation lengths (Table 8.14).

There are also species differences in the rate at which different regions of the stomach develop; glandular development is faster in the fundus than the antrum in man (0.15

compared with 0.25 gestation), while the converse is true in the rabbit (1.0 compared with 0.61 gestation) (Deren, 1971).

The histological demonstration of gastric enzymes in the fetal stomach is made difficult by the apparently different characteristics of some of these enzymes before birth. For example, although granulation is present in human fetal chief cells in the late first trimester, its staining characteristics differ from adult pepsinogen (Deren, 1971). The same is true for human fetal D cells (gastrin-containing cells; de Lemos, 1977). It is possible that such differences may partly account for the wide spread of gestational age across which chief cell and parietal cell secretion is stated to occur (Table 8.14). It seems likely that the neutral gastric pH until close to or even after term (see below), may explain the differing characteristics of the enzymes.

The intestinal epithelium is initially stratified, with columnar epithelium developing by the end of the first third of gestation in man (Kelley, 1973) and the sheep (Trahair and Robinson, 1986). Microvilli develop roughly in synchrony with columnar epithelium, together with mitochondria and Golgi bodies. Dense bodies and vesicles ('meconium corpuscles') which may be primitive lysosomes appear in the villous epithelial cells at much the same time (Andersen *et al.*, 1964). The rates of cell turnover and migration in the small intestine appear slower *in utero* than in adulthood in some species (rat, human, sheep) but not in others (pig, guinea pig) (see Grand *et al.*, 1976; Trahair and Robinson, 1986). The colon develops in parallel with the small intestine.

(b) Function before birth

The function of the gastrointestinal tract falls into three broad phases: intrauterine, during which minimal, if any, nutritive function is served; neonatal, during which a single, entirely specific food must be ingested and digested; and infant and adult, when weaning and maturation of function to deal with adult foodstuffs occur. Thus, the fetal gastrointestinal tract has, as it were, a breathing space after birth before the full complexities of adult feeding are thrust upon it.

Swallowing

The fetus shows episodic swallowing movements surprisingly early in gestation. The human fetus in the early second trimester can apparently swallow some 2–7 ml/day, a volume which increases with gestation age (Pritchard, 1965; Abramovich, 1970) to some 450 ml/day at term with substantial individual variation. The fetal lamb, studied directly with implanted flow probes, swallows a very similar daily volume by term (Mistretta and Bradley, 1975), although estimates made by a less accurate tracer dilution technique suggest considerably greater daily swallowed volumes (Tomoda *et al.*, 1985). It is fascinating to conjecture whether the fetus can taste what it swallows. Morphologically mature taste buds are present in man at about one-third and in the lamb from about two-thirds of gestation (Bradley and Mistretta, 1973), while in species of substantially shorter gestation, such as the rat, mature taste buds do not appear until after birth (1.52 gestation, Farbman, 1965). The application of various taste stimuli to the tongue of fetal lambs in the last third of gestation is accompanied by consistent responses in fibres of the chorda tympani, which does suggest that the fetus may be able to sense (although not necessarily perceive the 'taste' of) its surrounding liquor.

Protein digestion

Work by Gitlin *et al.* (1972) suggested that the human fetus could digest proteins present in amniotic fluid, and it was therefore suggested that the fetus might derive some of its nutritional requirements from swallowed liquor. Infants with oesophageal atresia

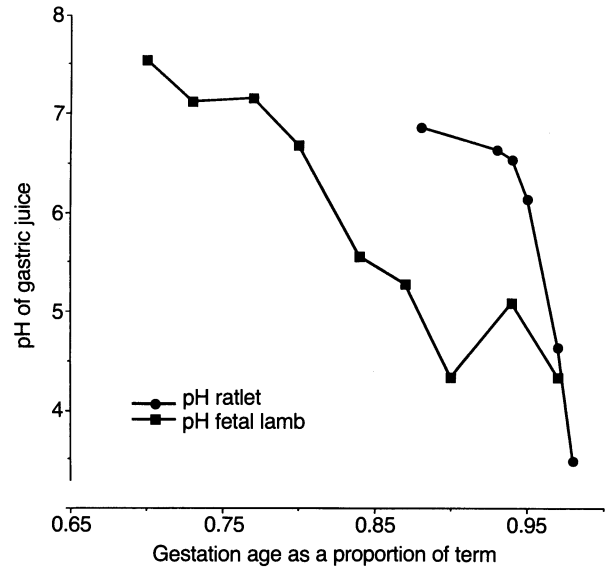

Figure 8.20 The pH of the gastric contents falls markedly in late gestation in the fetal lamb and rat, but does not reach adult values until after birth. (Data from Shulkes *et al.*, 1985, and Garzon *et al.*, 1981.)

tend to be smaller than normal infants. Oesophageal ligation in fetal rabbits resulted in reductions in both birth weight and crown–rump length (Mulvihill *et al.*, 1985), but when fluid was continuously infused into the fetal stomach after ligation no such effect was seen. These authors suggested that swallowed liquor might account for 10–14% of the fetuses' daily calorie intake, a figure similar to that suggested for the human fetus by Grand *et al.* (1976) and for the rhesus monkey fetus (Pitkin and Reynolds, 1975). In passing, it is perhaps interesting to note that the consumption of carbohydrates and amino acids across the intestinal circulation of the fetal sheep is such that it appears that nutrients derived from the amniotic fluid may in fact be being used by the intestine for its own metabolism and growth, rather than making any general contribution to the fetal nutrient pool (Charlton *et al.*, 1979a). It has also been suggested that early exposure of the gut to various proteins might be a requirement for gastric enzyme induction (e.g. Pitkin and Reynolds, 1975) and, furthermore, that the amniotic fluid contains unspecified gastrointestinal trophic factors, but this remains to be confirmed.

The fetus acquires its amino acids by placental transfer, and the need for protein denaturation *in utero* is thus minimal. Gastric acidification, which denatures proteins and stimulates pepsin activity for their digestion, is a feature only of late gestation. The functional ability for acid secretion is, however, present before it is physiologically expressed and appears to parallel parietal cell development (Wright, 1963). Figure 8.20 shows the temporal changes in the pH of the gastric contents of the fetal lamb and rat towards term, and it will be seen that, even at term, the gastric pH is substantially higher than that in the adult (Garzon *et al.*, 1981; Shulkes *et al.*, 1985).

This is a universal finding, and may partly be due to the buffering effect of ingested amniotic fluid.

Animal experiments suggest strongly that the control mechanisms for gastric acid secretion differ in fetus and adult.

Immunoreactive gastrin is present in the fetal stomach from at least halfway through gestation in man (see Grand *et al.*, 1976) and two-thirds through gestation in rabbits (Deren, 1971), but in addition to the basally low gastric acid secretion the acid secretory response to pentagastrin is both slow and small before birth (e.g. Garzon *et al.*, 1982; Shulkes *et al.*, 1985). This, in conjunction with the high plasma gastrin concentrations *in utero* (Shulkes *et al.*, 1982) and at birth (Euler *et al.*, 1977), suggests receptor immaturity, either in avidity or number, or the presence of a competitive inhibitor.

Gastrin administration to mature animals stimulates DNA and RNA synthesis and promotes the growth of the pancreas. The hypergastrinaemia of late gestation, with no negative feedback control via low gastric pH, may thus be exerting a trophic effect on the fetal digestive tract.

In the adult, the stimulatory effects of gastrin, histamine and acetylcholine on gastric secretion appear to be closely related, and it has been suggested that histamine may provide a final common pathway. This is not so in fetal rats or lambs, in which the responses to pentagastrin, carbachol and histamine, which begin to appear in late gestation, are entirely independent of each other (Garzon *et al.*, 1982; Shulkes *et al.*, 1985), as also seems to be the case in the newly born piglet (Forte *et al.*, 1975). Both histamine and vasocative intestinal peptide stimulate cAMP in gastric glands from human fetal stomach in the early second trimester (Gespach *et al.*, 1981), but pentagastrin administration was without effect in a proportion of infants born in the early third trimester (P.E. Hyman *et al.*, 1985).

Prostaglandin E_2 (PGE_2) exerts a cytoprotective effect on the mucosal cells in the adult, by its effect on sodium transport and mucus production. Little is known of its role in this respect before birth, but gastric PGE_2 concentrations were unmeasurable in infants delivered before the third trimester (Marino *et al.*, 1983). An adult-type correlation between gastric pH and PGE_2 concentration was not reached until 37 weeks' gestation.

Pepsin secretion may antedate (lamb), coincide with (rat) or follow (rabbit, pig) the onset of gastric acid secretion (Deren, 1971). Where its secretion develops post-natally, intestinal antibody transfer (see below) will be possible until such secretion develops. The optimum pH for pepsin activity is 1.8–3.5, so the high pH of the gastric contents *in utero* and in the immediate perinatal period will inhibit its activity. Pepsin secretion occurs relatively late in man (approximately 0.86 gestation), which could theoretically impair digestion in the premature newborn. However, the very process of birth, and gastric distension through feeding, have substantial effects on gastrointestinal functional development (see below).

Intrinsic factor appears to be synthesized by the parietal cells from the time of their differentiation (see Grand *et al.*, 1976). Its function *in utero* has not been studied and its secretion only increases slowly after birth (Deren, 1971).

Peristalsis in the human small intestine is demonstrable in the late first trimester, at which time beta-adrenergic receptors are already present (see Grand *et al.*, 1976). Alpha-adrenergic receptors are only demonstrable somewhat later, and appear to be anatomically differently sited. The prominent microvillus border, with marked ATPase activity, suggests the possibility of some absorption during fetal life, from at least a quarter of gestation (Lev *et al.*, 1972). The development of the brush border enzymes such as disaccharidases, peptidases and alkaline phosphatase is rapid from this time (Menard and Arsenault, 1985), with potential functional differentiation of different segments of the small intestine from 0.28 gestation. Thus, at 0.28 gestation glucose transport is similar in jejunum and ileum, but by 0.40 gestation such transport is greater in the jejunum (Koldovsky, 1970).

Macromolecules such as ferritin and horseradish peroxidase can be taken up from small intestine by the end of the first trimester in the human (Moxey and Trier, 1979) and in the rhesus monkey (Lev and Orlic, 1973). These villous absorptive cells resemble those of the adult in primates by approximately halfway through gestation, whereas a similar appearance is not found until weaning in the rat (Moxey and Trier, 1979).

Carbohydrate digestion

The intestinal digestion and absorption of carbohydrates before birth has been most widely studied in the chronically cannulated fetal lamb. Ruminant gastrointestinal physiology is obviously markedly different from that of other animals, so that even more caution than usual is required for between-species extrapolation. The response to duodenal instillation of glucose increased from late gestation to term, in terms of absolute blood glucose concentration reached, change from basal blood glucose concentration and the time to peak response and return to basal levels (Charlton *et al.*, 1979b). Lactose administration had similar age-related effects, while maltose and sucrose were without effect on blood glucose level at any gestational age. The smaller, slower effects in younger fetus probably relate to lesser absorption across the gut, either because of the smaller surface area or because of lesser activity of a glucose carrier. Maltase and sucrase are evidently absent in fetal lambs, by contrast with the human fetus, in which both are abundant in the third trimester (Lebenthal and Lee, 1980). Fructose absorption is by facilitated diffusion, rather than active transport, and is slow from the fetal lamb gut, regardless of gestational age (Charlton *et al.*, 1979b). In these experiments there was a good overall correlation between the change in blood glucose and that in lactate, the lactate presumably accumulating in the absence of other pathways for pyruvate metabolism.

Similar experiments performed acutely in the rhesus monkey in late gestation also showed a blunted response by comparison with adults, with, under these circumstances, no change in plasma insulin, growth hormone or glucagon (Chez *et al.*, 1974). Interestingly, infant monkeys delivered prematurely over the same gestation range, studied 4–5 h after birth, showed a much enhanced response in blood glucose concentration, and some increase in plasma insulin concentration.

Fat digestion

Fatty acids are transported across the placenta, and are then used as 'building blocks' for more complex lipid structures by the fetus. The functional demands for fetal intestinal lipid absorption must be small, but there is evidence for some intrauterine enterohepatic circulation for biliary lipids. Lipid can be absorbed from human fetal small intestine by 0.25 gestation, while biliary lipid secretion is present at least by halfway through gestation (see Grand *et al.*, 1976). However, the bile salt concentration is too low *in utero* to allow the solubilization of lipids, so although pancreatic lipase activity is present from approximately 0.4 gestation in rat, lamb and human fetus (Koldovsky, 1970), it may be functionally unimportant. Lingual and gastric lipase activity is discussed briefly below in relation to digestion in the newborn.

In the human embryo, by 4 weeks after conception two pancreatic buds have appeared as outgrowths of the lower end of the foregut. By 7 weeks, these buds have rotated and fused to give the appearance of the definitive gland and the pancreatic duct has formed. Although the human pancreas is anatomically and histologically mature well before birth, enzyme secretion is still immature. Differentiation of acinar cells takes place from 10 to 14 weeks and endocrine cells appear from 8 to 12 weeks. Pancreatic secretion does not begin until around the fifth

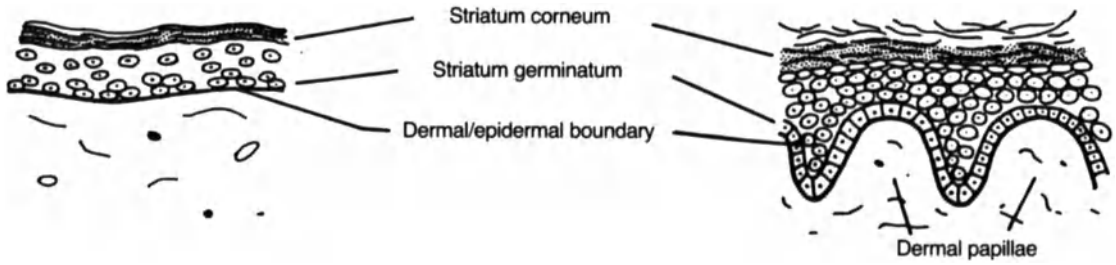

Figure 8.21 Transverse sections through the skin of human fetus of 28–30 weeks' gestation (left-hand panel) and one at term (right-hand panel). Note the development of the stratum corneum, with desquamation of the outermost epidermal layers by term, and the development of the dermal papillae.

month, and enzyme activity is detectable in human fetal pancreatic tissue from about this time. Trypsin, chymotrypsin and carboxypeptidase appear first in the human fetus, to be followed by enterokinase and pancreatic lipase (McClean and Weaver, 1993). However, even by term the levels of the last two are still less than 20% of the levels achieved in childhood. Enterokinase is necessary for the conversion of trypsinogen to trypsin, which in turn activates chymotrypsin and carboxypeptidase. Pancreatic amylase is not detectable in significant amounts until after birth, but human milk contains some amylase, which may aid digestion of starch at the time of weaning (Dewitt *et al.*, 1990).

The ontogenesis of gastrointestinal and pancreatic enzyme systems forms a substantial subject in itself, and is not further considered here. The interested reader is referred to the reviews by Grand *et al.* (1976) and Saraux and Girard-Globa (1982). Pancreatic endocrine function is considered in the review by Van Assche and Hoet (1984) and in section 8.2.2 in relation to glucose metabolism.

8.3.8 THE SKIN

The most important functions of the skin in extrauterine life are to act as a barrier to prevent the loss of water and electrolytes; to form a protective layer against the entry of such hazards as bacteria; and to provide a degree of insulation against extremes of environmental temperatures. The barrier properties of the skin lie in the stratum corneum, with its flattened, anuclear cells and high keratin content, bound together by a lipid layer with a specialized 'waterproofing' ceramide, polyoxyacyl ceramide (Nugteren *et al.*, 1985). However, the stratum corneum develops relatively late in gestation, so that the barrier properties of fetal skin are poorly developed by comparison with those of adults.

(a) Structure

The early development of the skin is very similar in all species. There is an initial single-layered ectoderm (primordial epidermis) and a single-layered mesoderm (primordial dermis). By 0.2–0.38 gestation (man, mouse) the germinative ectoderm has produced a single-layered periderm which covers the entire surface (Sengel, 1976; Holbrook and Odland, 1980). The germinative layer will also ultimately give rise to the strata spinosum, granulosum and corneum (Figure 8.21).

By the end of the first trimester in man the periderm is beginning to thicken in the plantar and palmar regions, and keratinocytes are distinguishable beneath it. These are also of ectodermal origin. Keratin proteins are found in fetal rabbit epidermis at 0.39 gestation, considerably before morphological differentiation of the epidermis has occurred, but are

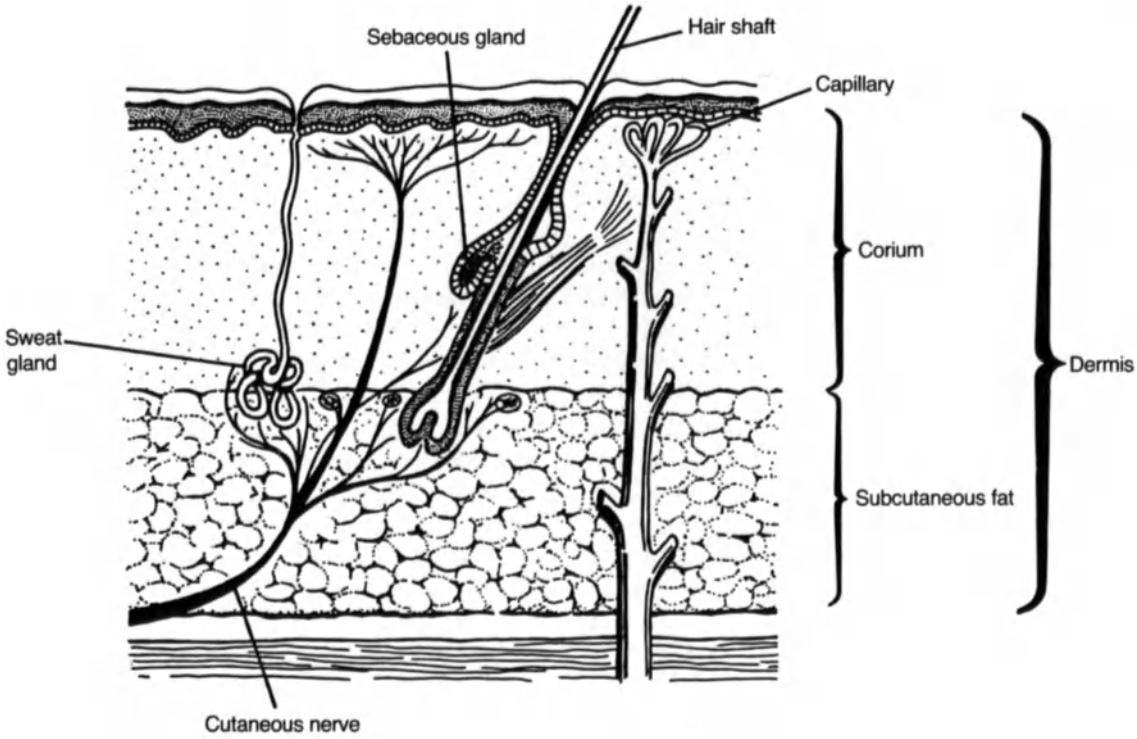

Figure 8.22 A transverse section through the skin of a term human fetus to show the epidermal appendages.

not present until much later (0.77) in the rat (Green and Couchman, 1984). There is a form of fetal keratin, of rather lower molecular weight, which disappears in late gestation in the rabbit (Banks-Schlegel, 1982). Interestingly, this is the keratin synthesized by the adult human conjunctiva. The development of a stratum corneum is dependent on synthesis of higher molecular weight, adult-type keratins (Banks-Schlegel, 1982). Full keratinization is first achieved in the nails (0.40 gestation, human) but epidermal keratinization is not complete until 0.7 gestation (Weston, 1985). As the stratum corneum forms, so the outer periderm is sloughed off, but this can occur at any stage from mid-gestation (man) to post-natally (mouse, Holbrook and Odland, 1980).

The dermis forms from mesenchyme, and is initially poorly differentiated. It is however, absolutely necessary for the normal development of the epidermis. It is identifiable from about a quarter of the way through gestation in the human and pig fetus, but not until halfway through in mice (Sengel, 1976). Differentiation into the two adult layers is fairly slow, and the subdermal fat layer develops still later.

A variety of epidermal appendages (Figure 8.22) are formed during fetal life, including the structures of the hair follicle, the sebaceous glands, the apocrine and eccrine sweat glands and the nails.

Hair follicles begin as downgrowths from the basal layer at the end of the first trimester in man and monkeys (Bressler and Munger, 1983) but much later in rats (0.73, Green and Couchman, 1984). However, while in man

subsequent development to actual hair formation takes several weeks, the same progression occurs in 4 days in fetal rats (Green and Couchman, 1984). It has been suggested that epidermal growth factor (EGF) is implicated in the development of hair follicles, since numerous receptors for EGF can be demonstrated over the outer root sheath and the epithelial cell layers at the follicle base (Green and Couchman, 1984). Actual hair formation begins on the scalp and proceeds cephalocaudally, and the hair follicles themselves are directed backwards. Animals which have whiskers (vibrissae) start to develop these in parallel with normal hair follicles. Small nerve plexuses, probably of Schwann cells, are found by halfway through gestation at the base of vibrissae follicles in mice (Bressler and Munger, 1983), and it seems probable that vibrissae only develop from such early innervated follicles. Animals with hairy skin use hairs as mechanosensitive receptors, and here again innervation develops relatively early (about 0.40 gestation in the rhesus monkey, Bressler and Munger, 1983) with circularly arranged afferent nerve terminals apparent soon after differentiation of the hair follicle.

The initial hair formed by the human fetus is the fine, silky lanugo hair, which resembles the vellus hair of post-natal life. Lanugo hair may be very prominent on the forehead and sides of the face, as well as the scalp, but by about halfway through gestation the follicles on the forehead start to involute and vellus hair formation begins, initially at the eyebrows and scalp (Holbrook and Odland, 1980). Wool-bearing animals do not develop lanugo hair (Thorburn *et al.*, 1981) and the primary wool fibres only become evident in late (> 0.8) gestation.

Sebaceous glands form from the epidermal downgrowths which result in the hair follicles and are first evident in human fetus at the end of the first trimester. They are active *in utero*, with high concentrations of hydroxysteroid dehydrogenases present by 0.4 gestation (Weston, 1985). They are particularly responsive to the high levels of androgenic steroids which are encountered in the fetal circulation and secrete lipid-filled cells in increasing numbers into the amniotic fluid as gestation progresses. This secretion is presumably an important component of the protective vernix caseosa (see below).

The eccrine sweat glands also form from downgrowths of the basal layer. The terminal coils of the sweat gland gradually increase in complexity, but the duct can be patent considerably before the adult-type structure is reached.

In man, the Pacinian (deep pressure receptor) corpuscles are fully formed at term, but Meissner's touch receptors are not (Maiback and Boisits, 1982). Interestingly, the function of the Merkel cells, which are simple touch receptors in the adult, may be different *in utero*. It has been suggested (Moll *et al.*, 1986) that they subserve a neuroendocrine function, secreting a tissue hormone(s) which promotes or induces the local formation and growth of such special structures as the hair follicles, eccrine sweat glands and/or nerves. They appear in the epidermis at the end of the first third of gestation and migrate into the dermis. However, by late gestation their distribution is again confined to the epidermis, suggesting that their dermal function is complete.

The hormonal factor(s) which regulate differentiation of the skin and its glands and appendages are not well defined. Certainly, EGF receptors are demonstrable in the last third of gestation in the areas of fetal rat skin in which rapid epidermal growth is occurring (Green and Couchman, 1984) and the number of receptors falls in parallel with the rate of mitosis. The administration of large doses of EGF to fetal lambs in the last quarter of gestation also evokes a marked hypertrophy of epidermal glandular structures, and increased skin wrinkling, but the changes in the primary wool fibres are degenerative in nature (Thorburn *et al.*, 1981). Furthermore,

the urinary excretion of EGF is low in neonates, whether lambs (Thorburn *et al.*, 1981) or humans (Evans and Rutter, 1986a).

(b) Permeability

The composition of amniotic fluid in early pregnancy closely resembles a dialysate of fetal and/or maternal serum (see above). It can be shown that the permeability of fetal skin to tritiated water before keratinization has occurred is very similar to that of chorion laeve or amnion (Parmley and Seeds, 1970). It thus seems likely that in early gestation both water and solutes can pass freely across the fetal skin. However, as gestation progresses, and the fetal kidneys begin to excrete urea into the liquor, the need to avoid osmotic dehydration becomes pressing. As soon as keratinization is initiated, the skin's permeability to water begins to drop dramatically, and with the attainment of full keratinization water diffusion detectable *in vitro* effectively ceases (Parmley and Seeds, 1970).

There has been considerable interest in transepidermal water loss after birth, since in the human newborn epidermal development is not complete until 0.85 gestation, and an increasing number of babies younger than this now survive and need nursing. Even at term, skin water losses are considerably higher than those of adults for the first few hours after birth (Rutter and Hull, 1979b). This is largely due to immaturity of the stratum corneum, but may also be influenced by the low serum albumin concentration normal at birth (Hammarlund *et al.*, 1984). Anyone who compares an infant delivered at less than 28 weeks (0.7) with a term neonatal mouse will see the similarities in skin colour and texture, with a thin, moist pink skin, with prominently visible blood vessels. The degree of keratinization is also similar, and in a dry environment skin water loss may be up to 10 times higher than that in adults (Harpin and Rutter, 1983). However, the neonatal mouse survives because of the high humidity in the nest, and humidification of the incubators used for nursing the very premature infant is a recent development, in which clinical practice has learnt from nature.

In both mouse and man, exposure to the extrauterine environment results in a very rapid development of the stratum corneum, with corresponding falls in water loss through the skin (Harpin and Rutter, 1983). This 'speeding-up' occurs even when delivery has been very premature indeed. Another consequence is that the evaporative heat loss, due to high skin water loss, is reduced. A further consequence of the high skin permeability before the stratum corneum has developed might be turned to use for the very premature human newborn. Experiments conducted 23 years ago showed that if previable human and mouse fetuses were immersed in fluid-filled incubators at 35°C with liquid oxygen tensions of 250 p.s.i., they could survive for at least 23 h (cited in Gleiss and Stuttgen, 1970). From this observation arose the concept of enhanced percutaneous respiration in the newborn, a concept recently supported by studies of human infants born between 25 and 41 weeks' gestation. In very premature infants in which the stratum corneum was poorly developed, oxygen and carbon dioxide were transferred up to 11 times faster than in term infants (Evans and Rutter, 1986b). It can be calculated that the very premature human newborn could obtain up to 20% of its total oxygen requirements transepidermally (Cartlidge and Rutter, 1987). The skin of such infants is also much more permeable to drugs and agents such as phenylephrine (Harpin and Rutter, 1983). This might provide an extra route of administration such as is being tested in adults for the continuous administration of drugs to combat angina. Experiments with fetal lambs suggest that drugs may also be absorbed from the amniotic fluid across the fetal skin (Mears and Van

Petten, 1977), but it has not been determined whether this might be of clinical benefit.

(c) Temperature regulation

The adult's skin is an important regulator of body temperature, both through variations in the blood flow to the cutaneous vasculature and by heat loss through eccrine sweating. The importance of sweating in this respect varies substantially between species, in that adult mice, rats, guinea pigs, rabbits and dogs hardly sweat at all, whereas men, cattle and horses sweat strongly (Precht *et al.*, 1973). Even at term, however, the human newborn is comparatively resistant to sweating, with a considerably higher threshold than adults. Infants of less than 32 weeks' gestation are apparently unable to sweat at all (see Maiback and Boisits, 1982). Term infants can respond by sweating to both acetyl choline and adrenaline given intradermally, but this ability develops late in gestation, and is not found at 0.9 gestation or less. As with transepidermal water loss, exposure to the extrauterine environment accelerates the development of eccrine sweating, but at a considerably slower rate.

Eccrine sweating, induced by increased ambient temperature, is under hypothalamic control. The so-called 'emotional' sweating, induced by stressful events, involves a higher centre in the premotor cortex, and its development appears to be much more tightly tied to post-conceptional age. The only species in which its development has been studied in any depth is man. It is only apparent in the human newborn after 36–37 weeks' gestation, regardless of whether the baby was delivered before this time or not (Harpin and Rutter, 1982), and by 40–41 weeks the response induced by a painful stimulus is similar to that in adults.

8.4 RESPONSE TO BIRTH

8.4.1 THE ONSET OF BREATHING

The immediate requirement of the independent newborn is to establish an alternative supply of oxygen. The first step is to open the lungs and form a functional residual capacity.

When the fetus emerges from the birth canal it has already experienced many powerful physiological stimuli, in part a consequence of the labour on the mother, but in part due to the effect of uterine contractions on the placenta and fetus itself. A noxious stimulus which might stimulate a gasp *in utero* would have little permanent consequence for only a relatively small volume of fluid would be drawn into the lung. Once the head is surrounded by gas then a stimulus that provokes a gasp usually leads to a sequence of events which is irreversible. The pulmonary adaptation to extrauterine life is completed rapidly in healthy, vaginally delivered infants but is somewhat slower if the infant is born by Caesarean section (Palme-Kilander *et al.*, 1993).

Under normal circumstances the newborn gasps within seconds of birth. Precisely what causes this response is uncertain, but it may well be the consequence of a crescendo of novel stimuli, including cold air, particularly on the face, the movement of limbs and joints into positions they have never been in before and the contact with new and different surfaces. Many mammals lick the faces of their young and in so doing remove fetal membranes, clear away debris and provoke respiratory responses. The whale, which delivers under water, immediately turns after the birth to follow the newborn as it dashes to the surface to take the first breath. Whales have been observed to toss their young repeatedly out of the water, which may well assist in the initial aeration of the lungs. In man, infants slow to take that first gasp have, over the centuries, been subjected to a range of stimuli aimed at provoking a first deep respiration,

from anal dilation to a splash of champagne on the face. Dangling them by their heels and slapping them on the back must be a particularly painful experience and is unnecessary; nothing seems to stimulate a gasp more effectively than blowing cold oxygen around the nose and mouth, and this has the advantage that when the gasp does occur an oxygen-rich mixture is drawn into the lungs.

There are a variety of reasons why newborn infants might fail to gasp at birth, from intrauterine asphyxia to congenital abnormalities, but the management of such matters is outside the subject of this chapter (Roberton, 1986). So too are the mechanisms which invoke gasping in asphyxiated newborns; it is probable that only in such situations does hypoxia or hypercapnia act as a stimulant. It is not necessary to postulate that the 'normal' infant is asphyxiated by the birth process and that asphyxia is a requirement for stimulating the initial gasp. Fetuses experimentally asphyxiated *in utero* do make gasping efforts, and babies born after intrauterine asphyxia often have vernix and meconium in their lungs, indicating that they must take deep respiratory efforts prior to birth. However, anyone who has attempted to study the exteriorized fetus close to term knows well that even minimal handling or a hint of cool air on the face provokes a gasp. Given modern anaesthetic techniques, infants born by elective Caesarean section are not hypoxic at delivery, the cord is pulsating freely and yet the majority gasp immediately with handling and clamping of the cord.

(a) The consequence of the first gasp

With the first gasp, air is drawn into the lung and the liquid–gas interface disappears down the respiratory tree. Such would be the surface forces that, despite the strength of the respiratory effort, that interface would not travel very far down the finer divisions of the airway if it were not for a natural detergent, 'surfactant'. This is a phospholipid secreted by the type II pneumocytes lining the air sacs, and it is the properties of this chemical that permit the air to be held in the terminal air sacs and alveoli to form the reservoir, the residual volume, which is essential for gaseous exchange (J.A. Clements, 1977). Natural or synthetic exogenous surfactant, used to improve lung function in immature infants who lack endogenous surfactant (OSIRIS Collaborative Group, 1992), probably helps by increasing functional residual capacity rather than increasing lung compliance (A. Milner, 1993).

With the first inspiratory effort, air usually enters the lungs and with expiration some is retained. But this is not inevitably so (A.D. Milner and Vyas, 1982). The formation of the functional residual capacity does not always follow the first inspiration. Examples of pressure–volume loops during the initial respiratory efforts of three infants are shown in Figure 8.23.

In Figure 8.23a, as the intrathoracic pressure falls, air is drawn in to a volume of 60 ml. With expiration air leaves the lungs until 20 ml is left as a first contribution, as it were, to the formation of the residual volume. With the second inspirating effort 80 ml is drawn in, and at the end 40 ml remains in the lung. The residual volume forms in steps. This is probably the usual pattern. In human infants the functional residual capacity (40 ml/kg) forms to about 80% of its final volume in the first few minutes.

However, there are wide variations in pattern and timing; this can be easily appreciated by watching the respiratory movements of newborns at birth. For example, some with their first efforts draw very little air in. This, along with studies on isolated lungs, led to the concept of an 'opening pressure'. By this is meant that a certain negative pressure had to be generated before any air entered the lung, and then air 'rushes in'. The opening pressure phenomenon is the rule when collapsed isolated lungs are inflated via the trachea. It may occur under

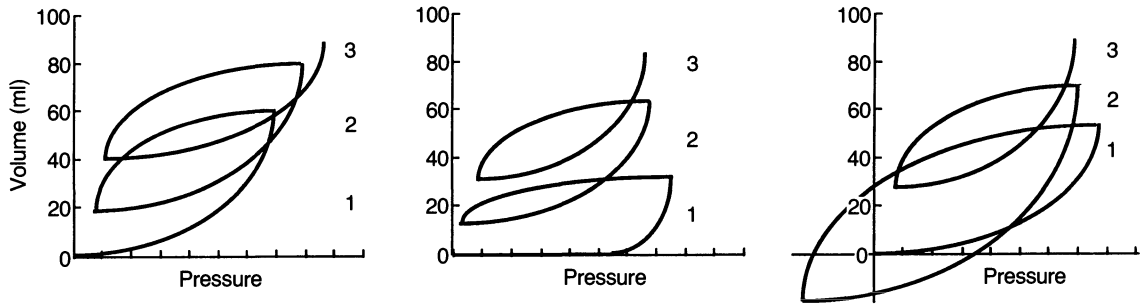

Figure 8.23 Stylized pressure–volume curves of the first three respiratory efforts. The first shows a rapid stepwise formation of a residual volume. The second illustrates an opening pressure. The third show and infant expelling gas which had entered due to recoil of the chest cage after birth.

normal circumstances, but whilst in the former it probably relates to surface characteristics in the latter 'the opening pressure' is more likely to be due to upper airway obstruction.

(b) The formation and maintenance of the functional residual capacity

A third sequence of events is shown in Figure 8.23c. Here the first gasp draws in air but it all leaves again; indeed more leaves than was inhaled! This clearly demands an explanation. Whilst surfactant permits gas to be held in the alveolar sacs with the gas–liquid interface lining the type I and II pneumocytes, it will not, of itself, retain gas in the lungs. That demands other forces, namely the shape of the chest wall and the tone of the diaphragm. For more to leave than is inhaled implies that air has entered without obvious inspiratory effort.

In section 8.3.3(c) the formation and circulation of lung liquid was reviewed. The volume of lung liquid in sheep is 40 ml/kg, which is close to the functional residual capacity after birth. It would seem that the thorax and diaphragm in its resting state *in utero* hold a volume of fluid equivalent to the volume of gas the lungs hold after birth. There is some evidence in sheep that this lung liquid begins to clear from the lungs in preparation for birth. However, at birth in man, and presumably other mammals born head first, the thorax is squeezed in the birth canal. Not infrequently, liquid can be seen pouring out of the nose and mouth. When the thorax emerges from the birth canal it may well resume its initial shape and thus air is drawn passively into the lung. Various attempts have been made to estimate this volume; in man, it can be as much as 40 ml, but it is very variable.

Other mechanisms which have been postulated have been 'frog breathing' by gulping air, and priapism of the pulmonary vasculature, thus splinting the lungs open to hold gas. At the present there is little evidence to support either idea. It seems far more likely that the formation of the functional residual volume depends primarily on the tone and position of the diaphragm, and this may well change prior to the first gasp. Similar sequences can be seen in the limb muscles as they pick up tone and begin to move after birth.

Once the first gasp has been made, then the functional residual capacity will be held in part by air trapping, but also by the tone and position of the diaphragm and chest wall. Once a functional residual capacity has formed, and only when it has formed, will tidal respirations, breathing, commence.

After birth, with the opening of the pul-

monary circulation (section 8.4.2), lung liquid is drawn from the lungs probably in the main via the capillaries and veins, and at a slower pace via the pulmonary lymphatics. This too results in a passive formation of a residual volume, but on a different time scale, and may well be responsible for the slow increase in functional residual capacity after the first few minutes and over the first few hours (for review, see Strang, 1977).

8.4.2 CIRCULATORY CHANGES AT BIRTH

Whilst the most dramatic action seen at birth is the newborn's efforts to expand its lungs, the event which has the most profound and widespread consequences is the sudden irreversible occlusion of the umbilical cord.

The cord may be occluded in the last stages of labour, it may snap at the birth, or it may pulsate for a while until some action, stretching or chewing (or, in man, clamping) causes the vessels to go into spasm. A whole variety of steps are taken by parents, usually mothers, to get rid of the 'afterbirth'. For herbivores it appears to be the only time they eat flesh – a meal rich in iron and long-chain polyunsaturated fatty acids amongst other things.

In human infants, many papers have been written on the desirability of early or late clamping of the cord (Oh *et al.*, 1966), and the position of the baby in relation to the placental bed, and the benefits or harm that may accrue from milking or massaging the cord. In animals who deliver whilst standing, the placental bed is higher than the fetus and the fetus may receive an additional flow of blood via the umbilical vein as it falls free of the birth canal before the vessels go into spasm. When a mother, lying on her back, is delivered by a Caesarean section, the infant is lifted out of the womb and blood can then flow from baby to placental bed. That obviously must be avoided. However, the advantages and disadvantages of early or late clamping appear to balance out, except perhaps in the preterm infant (Kinmond *et al.*, 1993). The newborn with extra blood has more problem with neonatal jaundice (Saigal *et al.*, 1972) but is less at risk of subsequent anaemia.

Occlusion of the cord leads to certain predictable events. The venous return via the umbilical vein ceases, thus the ductus venosus collapses, and from then on the portal vein is the sole supplier of the portal venous system. With the drop in flow through the inferior vena cava, the pressure in the right atrium falls and the foramen ovale closes, an event which is reinforced when the increasing pulmonary venous return increases the pressure in the left atrium. Also, within seconds, the chemoreceptors are perfused by blood drawn from the lungs rather than oxygenated blood from the placenta. With the clamping of the umbilical artery, a sudden increase in the peripheral resistance occurs and the blood pressure rises (Dawes, 1968).

Expansion of the lung leads to a fall in pulmonary arterial resistance and pulmonary flow increases. This happens even if the lung is filled with nitrogen. If it is expanded with air, oxygen is drawn into the body. This mechanical effect of lung inflation on pulmonary vasculature is reinforced by the effects of falling carbon dioxide, rising pH and rising PO_2, all of which favour pulmonary vasodilation, and thus lung perfusion is established, maintained and controlled. Hormonal changes may also influence pulmonary vascular resistance (Soifer and Heymann, 1984; Mott, 1985; Soifer *et al.*, 1985a,b; Kühl *et al.*, 1989; Davidson and Eldemerdash, 1990; Hargrave *et al.*, 1990) and there must also be receptors responsive to agents which do not occur naturally but have potent pulmonary vasodilator properties, such as magnesium sulphate and nitric oxide (Kinsella *et al.*, 1992). The sudden drop in pulmonary vascular resistance results in a reversal of the flow through the ductus arteriosus. Now oxygenated blood flows back to the left atrium, and is distributed by the left

ventricle, so that oxygenated blood flows back through the ductus. The ductus is a muscular vessel and its muscular wall is susceptible to PO_2 such that as the PO_2 rises the muscle contracts, a mechanism which involves prostaglandins. Drugs inhibiting the synthesis of prostaglandins cause the duct to close (Heymann *et al.*, 1976; Rennie and Cooke, 1992), whereas an infusion of prostaglandin E_1 can keep the ductus open (Hallidie-Smith, 1984). With muscle spasm, the ductus closes 'physiologically' within a matter of minutes of birth but at this time, with an episode of asphyxia, it will open again. However, over a matter of weeks the closed ductus slowly, seals up anatomically and is replaced by a fibrous cord.

With the fetal channels closed, the ventricles now contract in series with equal stroke volumes. The systemic blood pressure rises and the pulmonary blood pressure falls. The systemic arterial PaO_2 also increases and the supply and uptake of oxygen to many of the organs of the body rises. In most mammalian species the resting metabolic rate at birth is relatively low and it gradually increases over the first days of life. However, the timing and magnitude of the rise varies from species to species, and in some little or none takes place. The reasons for the increase presumably reflect increasing activity in organs such as the bowel and liver but possibly, in those animals with advanced motor development at birth, increased muscular activity to maintain posture.

8.4.3 NUTRITION

A less urgent but equally critical consequence of interruption of the placental circulation is the withdrawal of a 24 h near-constant supply of glucose, amino acids and fatty acids. The glucose, being available and controlled by maternal haemostatic mechanisms, was the main source of cellular energy, amino acids were directed for structure and fatty acids for structure and energy storage. As we have seen in section 8.3.6(b), the energy reserves of glycogen and fat vary widely with species, and hence their capacity to survive in the face of total starvation.

Independent existence demands a number of adjustments. The most immediate is the maintenance of the blood glucose level. In all species studied, the already relatively low blood sugar concentration falls after birth to slowly rise again. The fall presumably reflects an uptake in excess of supply. The immediate source of supply is liver glycogen. Although this can be rapidly consumed in the face of asphyxial episodes, in most species under usual conditions it meets the body's glucose needs for some hours. Next, the liver accelerates gluconeogenesis from amino acids, ketones and fatty acids, and from any lactate produced as a consequence of asphyxia (Girard, 1986). Ketones themselves may be a significant alternative source of energy in some species (Warshaw, 1979; Robinson and Williamson, 1980). The enzymes involved in hepatic gluconeogenesis can be induced by catecholamines, glucocorticoids and pituitary peptides. Their activity is greatest in fetuses with poor growth rates before term (intrauterine growth retardation).

Species differences are important in the metabolic adaptations to the nutritional changes following birth. These changes have been most comprehensively studied in the rat. The rat fetus is largely dependent on a carbohydrate 'diet' (principally transplacental glucose) from the mother but, following birth, rat milk contains lipid as the predominant energy source. The amount of lipid intake then falls again when the rat pup is weaned on to an adult rat diet. In an elegant series of experiments (Girard *et al.*, 1979; Girard, 1986), marked hormonal and enzyme changes have been observed during these nutritional transitions from placental supply to suckling to weaning. These are necessary because the newborn rat has a greater requirement for glucose utilization per unit body weight than the adult animal, partly

because of the relatively larger brain size, but must achieve this despite a milk which is relatively lacking in carbohydrate. The adaptations involve the switch on of gluconeogenesis from amino acid substrates, the utilization of alternative fuels such as fatty acids and ketone bodies to spare glucose, and the inhibition of storage of glucose as glycogen. These changes occur very soon after birth, and it has been shown that the concomitant changes in the necessary enzymes, such as phosphoenolpyruvate carboxykinase (section 8.3.6b), are induced partly by the substrates in the diet. At weaning, there is a second switch in gene expression to promote the synthesis of enzymes which favour storage of glycogen as a protection against fasting (starvation being more likely in the foraging adult than the pup which has a ready supply of milk). These adaptations are most obvious in the rat, compared with the sheep or the human, which have less abrupt substrate transitions, but all mammals show similar, although perhaps less pronounced, responses following birth.

In utero, adipocytes are set to store fat and indeed take up free fatty acid from the circulation. After birth, this process is reversed, presumably in response to circulating catecholamines and sympathetic nervous activity, and there is release of fatty acids into the circulation for use elsewhere. Again *in utero*, peripheral tissues probably have a low rate of fatty acid oxidation as an energy source; the enzymes for fatty acid oxidation are present, but it may take time before they become fully functional (Warshaw, 1972; Roux and Myers, 1974; Zimmermann *et al.*, 1986). Certainly triglyceride droplets appear in many cells after birth from muscle to mucosal lining suggesting fatty acid uptake in excess of cellular capacity to use them. In contrast, brown adipocytes – at least in rabbits – do not appear to have this delay (section 8.4.6).

Whilst the internal adjustments are made, the newborn animal seeks an external source of nutrient. It is the peculiarity of mammals that their mothers supply a special complete food mix to meet all the nutritional requirements of their young. The physiology of this remarkable phenomenon is reviewed in Chapter 12.

8.4.4 THE GASTROINESTINAL TRACT IMMEDIATELY AFTER BIRTH

To obtain food the young must suckle. The physiological events involved in establishing digestion and absorption and the colonization of the bowel to assist in final digestion and excretion are as dramatic, though more leisurely, as those involved in establishing gaseous exchange (Weaver, 1992).

Suckling

Although the fetus swallows amniotic fluid, this is less of a challenge than suckling, when it is vital that milk is not aspirated in to the air-filled lungs. Coordinated sucking and swallowing requires competent neurological reflexes, laryngeal function and a degree of pharyngeal muscle tone that are only achieved in the human infant by 35 weeks' gestation (Herbst, 1989). This gestational age coincides with a significant increase in circulating concentrations of intestinal regulatory polypeptides (gastrin, motilin, neurotensin) in response to milk feeds (Lucas *et al.*, 1978). Therefore, unlike other systems which mature earlier in gestation and can function adequately, although not perfectly, following extremely preterm delivery (e.g. the kidney and endocrine pancreas), the gastrointestinal system cannot support independent nutrition until well into the final trimester.

Gastric acid secretion

Gastric acid secretion increases dramatically after birth in humans (Euler *et al.*, 1979) and guinea pigs (Hill, 1956), giving the gastric contents a pH compatible with pepsin

activity within 1–2 h. This process takes some 36 h in the lamb, and 21 days in the rat (Hill, 1956). Acid output per kg body weight is low at birth in all species, but once initiated rises rapidly. In the relatively mature newborn lamb and guinea pig it occurs whether or not the neonate is fed (Hill, 1956). Pentagastrin is without stimulatory effect on acid secretion in the newborn term human infant (Euler *et al.*, 1979) and basal acid output does not correlate with plasma gastrin concentrations. This is also so in infants delivered before 37 weeks' gestation (P.E. Hyman *et al.*, 1985), but in both there is a progressive increase in response to pentagastrin with post-natal age, regardless of post-conceptional age. Plasma gastrin concentrations are high at birth in most species except rodents, and fall slowly, presumably as the negative feedback loop from gastric pH is established and gastrin receptors mature.

Effect of feeding

Natural milk contains a least one growth-promoting hormone, epidermal growth factor (Oka *et al.*, 1983; Read *et al.*, 1984), which stimulates gastric and intestinal growth to a greater extent than isocaloric formula feeding. The presence of food in the stomach itself initiates surges in a variety of gastrointestinal hormones. This has been shown in comparisons of enterally and parenterally fed premature human infants (Lucas *et al.*, 1986). Plasma concentrations of enteroglucagon, gastric inhibitory polypeptide, motilin and neurotensin were all substantially increased by comparison with levels at delivery if enteral feeding had been given, but were the same or lower following parenteral feeding. Since both gastrin and enteroglucagon appear to be trophic hormones for gastrointestinal tissue, the clinical importance of this observation is apparent. In later-maturing species, such as the rat, weaning has a similar effect. Antral gastrin concentrations increase sharply at weaning in this species with parallel increases in intestinal wet weight and development and relative nucleic acid content. Forced early weaning accelerates all these processes (Herbst and Sunshine, 1969), while prevention of weaning delays it (Lichtenberger and Johnson, 1974). The administration of pentagastrin to rat pups which have been prevented from weaning stimulates the normal developmental changes (Lichtenberger and Johnson, 1974).

Immunoglobulin transfer

One of the most interesting consequences of differing gastric acidity, both between species and with post-natal age, is the effect on immunoglobulin transfer, and hence the acquisition of passive immunity. The guinea pig, relatively most mature at birth, receives maternal antibodies by placental transfer, with no post-natal intestinal absorption in the face of rapid gastric acidification (Hill, 1956). Lambs, foals and human infants take somewhat longer to achieve low gastric pH, and absorb antibodies from colostrum in the immediate post-natal period (Hill, 1956; Jeffcott, 1975; Grand *et al.*, 1976). Newborn piglets fail to acidify their gut contents to pepsin-activating levels until about 5 days post-natally, again coinciding with the period of colostrum absorption across the small intestine (A.A. MaçDonald *et al.*, 1982). The rat is the most extreme in this context, with highly selective uptake, transport and discharge of homologous IgG immunoglobulins in the proximal small intestine until day 22 post-natally (Deren, 1971; Rodewald, 1973; Murphy and Daniels, 1979). At this time 'closure' occurs abruptly, coincident with the onset of gastric acidification and weaning (Rodewald, 1973).

Carbohydrate digestion

The ability to digest different carbohydrates develops at different times in gestation (see above) but is adequate at term for sugars

characteristic of that species' milk. Thus, in man both lactase and sucrase are present and functional at term (Lebenthal *et al.*, 1983), whereas in the sheep, sucrase is not present at birth (Charlton *et al.*, 1979b). There is rapid post-natal maturation of the response to a glucose load, which appears to be dependent on post-natal, rather than post-conceptional, age (see Van Assche and Hoet, 1984).

Protein digestion

Protein digestion in the newborn occurs mainly in the proximal small intestine and appears to be adequate, at least in dealing with milk proteins. The intestinal concentration of trypsin in the human duodenum in the neonatal period approaches that of the adult (Lebenthal *et al.*, 1983). However, such widely differing species as man and the rat show very low duodenal concentrations of chymotrypsin and trypsinogen at this time (Saraux and Girard-Globa, 1982; Lebenthal *et al.*, 1983), which increase markedly in the post-natal period. The peptides and amino acids resulting from primary protein digestion are readily absorbed in the neonatal period, cytosolic peptidases peaking early and declining towards weaning (see Lebenthal *et al.*, 1983). Even in the neonatal rat, with minimal luminal protein digestion, the small intestine has an apparently functional active transport system for both neutral and basic amino acids, the neutral forms being preferentially transported (Murphy and Daniels, 1979). The proximal colon of the newborn pig, a species idiosyncratic in many ways, can also transport amino acids from its lumen to the circulation for the first few days (Jarvis *et al.*, 1977). Whether this extra site of absorption is related to their almost total retention of ingested nitrogen in early life (section 8.4.5) is not known.

Fat digestion

Fat digestion, conversely, is not especially efficient in neonates, presumably related to their low intraluminal concentrations of lipase, colipase and bile acids (Saraux and Girard-Globa, 1982; Lebenthal *et al.*, 1983). This is faintly surprising in view of milk's high fat content (up to 53% in the fur seal; Jenness, 1974), and the presence of a potent lingual lipase in the fetus and newborn, which can catalyse gastric lipolytic activity, may be a specific adaptation in response to these deficiencies. Furthermore, grossly raised intestinal concentrations of colipase, which protects lipase from rapid degradation, have been found in the neonatal rat, which may enhance the activity of such lipase as is present (Saraux and Girard-Globa, 1982). Again, the neonatal piglet is anomalous, with a considerable colonic capacity for lipid absorption during the first few post-natal days (Jarvis *et al.*, 1977), presumably necessitated by the low (7%) fat content of pig milk (Jenness, 1974).

It seems possible that the relatively poor lipid absorption of the neonatal period may adversely influence the absorption of the fat-soluble vitamins A, D, E and K, but the neonate has been born with placentally derived stores of these vitamins, so that deficiency is not usually encountered. Absorption of the water-soluble vitamin B_{12} in the newborn rat is highest in suckling and lowest in adult rats (Said *et al.*, 1985) and involves a carrier-mediated system. Serum B_{12} concentrations in the human are high at birth, but fall to a nadir by 30–40 days, only reaching adult values by 3–4 months (Grand *et al.*, 1976). This might be related to the substantial requirements for the vitamin in the face of low concentrations of intrinsic factor.

Ion absorption

Ion absorption in the immediate neonatal period seems not to have been widely studied, although species differences would be expected in view of the differing ionic composition of the milks. Iron uptake across

the duodenum has been found to be substantially higher in the newborn guinea pig than the adult, probably because all the villus enterocytes take up iron, rather than only those at the villus tips (Debnam *et al.*, 1987). It is of some practical interest that the gastrointestinal absorption of sodium in the human newborn is directly related to conceptional age, and that premature infants are thus at risk of sodium loss in the stools as well as in the urine (Al-Dahhan *et al.*, 1983b). The increasing gastrointestinal sodium absorption is probably mediated via aldosterone, as in the kidney.

Peristalsis and defecation

The neuromuscular development of the human gut occurs principally in the first trimester. Circular and longitudinal muscle are present in both small and large bowel by 10 weeks, and the autonomic neural plexi are identifiable by 13 weeks. These structures, necessary for peristalsis, are morphologically mature long before enteral feeding is required or coordinated sucking and swallowing possible (see above). The preterm human infant passes an average of one stool per day from as early as 25 weeks' gestation, even if not enterally fed (Weaver and Lucas, 1993). This suggests that there is an intrinsic pattern of large bowel motor activity which can function in a coordinated propulsive fashion from just after mid-gestation. This does not usually lead to defecation *in utero*, though what prevents prenatal evacuation is not known, meconium-stained liquor occurring only rarely before term (Lucas *et al.*, 1979; Mathews and Warshaw, 1979). At term, this is often not a normal event but, rather, occurs as a sympathetically mediated response to fetal distress.

The newborn infant has no voluntary control over evacuation, and defecation probably occurs as a reflex response to rectal load. Milk feeds entrain the intrinsic activity of the colon and induce regular defecation at a frequency determined by the volume of the products of digestion which reach the rectum; the more feeds, the more stools (Weaver and Lucas, 1993). Although the amount of stool varies, the water content remains within a narrow range (around 70%) even in preterm infants, suggesting that the water reabsorptive functions of the colon are present before term. After the first week of life, stool volume falls although milk intake continues to increase, partly due to a further maturation of the water-conserving capacity of the gut.

Although the fetus derives its nutrients transplacentally, nevertheless gastrointestinal development at birth is such that function can be immediately 'switched on', and in a way matched to the composition of the mother's milk. The widely differing ways in which individuals of differing species ultimately reach the common stage of weaned self-sufficiency is one of the many marvels of growth and development.

8.4.5 KIDNEY FUNCTION AFTER BIRTH

At the moment of birth, the fetus is abruptly removed from its dialysis unit, and must very rapidly run its own excretory function. It is helped in this by the fact of being in an anabolic state, so that requirements for urea excretion are low, and by the fairly low sodium content of milk. However, the low urea excretion itself influences the kidney's ability to concentrate the urine, and the low sodium content of milk can put in jeopardy those species such as the horse and man which can to some extent tolerate preterm delivery, since the premature foal and human are both at risk of moving into negative sodium balance (Al-Dahhan *et al.*, 1983a; Broughton-Pipkin *et al.*, 1984). The post-natal maturation of renal function is partly dependent on anatomical changes, but the importance of these changes varies from species to species. Thus in man, and in guinea pig, nephrogenesis is complete before term (Table 8.12; M.S. MacDonald and Emery, 1959;

Spitzer and Edelmann, 1971), while in the pig and rabbit nephrogenesis continues for up to 3 weeks post-natally (Leeson and Baxter, 1957; Broughton-Pipkin 1973; Friis, 1980). However, in all species there is an initial glomerular preponderance, with short proximal tubules and loops of Henle at birth.

The distribution of the cardiac output changes dramatically after birth, particularly in the renal circulation. Thus in fetal lambs some 3–4% of the combined ventricular output perfuses the kidneys, but shortly after birth this has risen to 8–10% (Iwamoto *et al.*, 1985) and similar or greater increases are seen in other species. This must suggest a fall in renal vascular resistance and such has been shown in the immediate perinatal period (e.g. Jose *et al.*, 1971; Spitzer and Edelman, 1971; Bailie *et al.*, 1980). The plasma angiotensin II concentration falls after birth (Robillard and Nakamura, 1988; Robillard *et al.*, 1988), and may partly account for this fall in renal vascular resistance; increased production of vasodilator prostanoids may also be implicated, as may the release of atrial natriuretic peptide, which antagonizes angiotensin (P.J. Harris *et al.*, 1987; Varille *et al.*, 1989; Stephenson and Broughton-Pipkin, 1990).

Glomerular filtration

The GFR rises after birth in all species studied at a rate too fast to be the result of purely anatomical change. For example, the GFR is nearly five times higher at the end of the first post-natal week in the lamb than in late gestation (Robillard *et al.*, 1980), while it doubles over the first week in the term human infant (see Aperia and Zetterstrom, 1982; Coulthard, 1985). The rise in the guinea pig is less dramatic, more maturation having taken place *in utero* (Spitzer and Edelman, 1971; Merlet-Benichou and de Rouffignac, 1977). The rise is still slower in piglets, which only increase their GFR by a third over the first 7 days (Friis, 1979), but there is an anatomical impediment in the shape of epithelial diaphragms over the glomerular fenestrae in this species.

Most of the rise in GFR is undoubtedly due to the rise in mean arterial pressure and fall in renal vascular resistance after birth, but these are not the only explanations. The hydraulic permeability of the glomerular membrane itself and the surface filtration area also increase with post-natal age (Spitzer and Edelman, 1971). There are also changes in the forces opposing filtration, as the colloid osmotic pressure and the hydrostatic pressure in the early proximal tubule rise, but these are more than counterbalanced by the rise in systemic arterial pressure and fall in renal vascular resistance.

Sodium

The distal tubular sodium reabsorption in the term newborn is highly efficient, a necessary corollary to the otherwise high sodium loss consequent on the short proximal tubules. This appears to be due to the very high plasma aldosterone concentrations found at birth (e.g. Beitins *et al.*, 1972; Giry and Delost, 1974) and results in a fractional excretion of sodium of less than 1% shortly after birth (e.g. Merlet-Benichou and de Rouffignac, 1977; Robillard *et al.*, 1977, 1980; Alt *et al.*, 1984), allowing the accumulation of the sodium needed for growth. The darker side of this lies in the inability of the newborn to excrete a sodium load (Dean and McCance, 1949; Goldsmith *et al.*, 1979), but this is unlikely to be of practical importance to species other than man in whom iatrogenic interference or over enthusiastic formula feeding may overload the baby's kidneys.

The marked rise in sodium reabsorption after birth is paralleled by a sixfold rise in renal oxygen consumption in the lamb and rat (Iwamoto *et al.*, 1985). Fetal kidneys can store glycogen, and it appears that the necessary enzymes for gluconeogenesis are present in the kidney at birth, since higher renal venous than arterial plasma glucose

concentrations can be observed post-natally (Iwamoto *et al.*, 1985). This ability presumably provides the kidney with an energy reserve to allow for the rapid increase in function during the period after birth and before suckling and digestion are initiated when circulating plasma glucose concentrations can fall fast (section 8.4.4).

Potassium

The fractional excretion of potassium rises sharply after birth (e.g. Robillard *et al.*, 1980), and this is also presumably a function of the high plasma aldosterone concentrations. Urinary acidification is not very efficient in the first few post-natal days (see Guignard, 1982), and this may partly reflect preferential exchange of potassium for sodium at the distal tubule. The neonatal kidney reabsorbs nearly all the filtered phosphate (McCance, 1972), and this will also inhibit hydrogen ion excretion. Furthermore, the renal threshold for bicarbonate is low at birth (see Guignard, 1982) so that urinary pH is relatively high at this time.

Water excretion

In most species the urinary osmolality is low at birth, although the mature guinea pig fetus can produce a hypertonic urine, thought to be due to its high plasma AVP concentrations (Merlet-Benichou and de Rouffignac, 1977). It appears however, that in other species the effector link between AVP and cyclic AMP may be immature at birth (see Joppich *et al.*, 1981; Siegel, 1982). Free water clearance is therefore reduced and water intoxication occurs more easily than in the adult (Tarnow-Mordi *et al.*, 1981; Vanapruks and Prapaitrakul, 1989). The osmotic threshold (the plasma osmolality above which AVP secretion is stimulated) is lower in term infants than in adults (Sujov *et al.*, 1984), but there is some evidence for a suppression of AVP with fluid loading in term human babies (Aperia *et al.*, 1984). The high prostaglandin production of the medulla will also inhibit the action of AVP. Furthermore, the urea-dependent concentrating gradient is very small in the medulla of the newborn kidney because of the rapid utilization of nitrogen by the growing tissues (see Siegel, 1982). The hypertonicity of the inner medulla is still further lowered by the decreased sodium reabsorption in the shortened ascending limb of the loops of Henle. The extracellular fluid volume is considerably greater per kg body weight in the newborn than the adult (Anthony *et al.*, 1992; Stephenson and Rutter, 1992a), and the initial 'failure' of concentrating ability allows the excretion of this excess fluid in the physiological diuresis and weight loss of the first few days. This diuresis is most marked in species such as the guinea pig, which are relatively mature at birth, and least in those born at a more immature stage, such as the rat, although even in the latter the ability develops rapidly after birth (D.P. Alexander and Nixon, 1961). Conversely, the inability of the newborn to excrete a concentrated urine means that it can become dehydrated considerably more easily than the adult, so that conditions such as scouring (diarrhoea) and vomiting are of greater clinical significance at this age.

Relatively little appears to be known about the effect of parathormone (PTH) on the neonatal kidney (Senterre and Salle, 1988), but since that kidney avidly reabsorbs virtually all the filtered phosphate it must be assumed either that plasma PTH is very low at this time or that PTH receptors are absent or non-functional (McCance, 1972). Conversely however, the kidney's ability to convert vitamin D_3 to the active 1,25-dihydroxycholecalciferol appears to be present well before term in man (McCance, 1972).

The kidney of the newborn mammal differs markedly in function from that of the adult, and yet we should not be too hasty in regarding this 'immaturity' as being disadvanta-

geous. Rather, it stems from the newborn's need for different patterns of solute reabsorption to allow for rapid growth and the excretion of the 'excess' extracellular fluid volume of the fetus (Shaffer, 1987; Simpson and Stephenson, 1993). It is only when for ethical or economic reasons we attempt to salvage the prematurely born human infant (Stephenson and Rutter, 1992b) or thoroughbred foal that the lack of integration of the facets of renal function before birth can become life-threatening (Wilkins, 1992).

8.4.6 COLD

Not only does birth result in occlusion of the cord so that the lungs must begin gaseous exchange, the bowel to absorb nutrients and the kidney control water and ions, the developing mammal also loses the protection of the uterus. *In utero*, the fetus is largely inactive and warm. With birth comes the freedom to move about. For some newborn it is a necessity if they are to survive. For example, the newborn whale must swim to get oxygen, the newborn caribou born on migration must stay with the herd. Exercise will put much greater demands on the cardiorespiratory system than those necessary to meet resting requirements. The fact that the newborn survive suggests that the cardiorespiratory reserves after birth are considerable.

A major metabolic challenge that all newborn experience is cold exposure. Except under experimental procedures the fetus does not experience cold, so in its broadest sense the systems for thermoregulatory control are untested. An outline of the nature of thermoregulatory responses is given in Figure 8.24 (Mount, 1979).

The environmental temperature at which mammals feel 'comfortable', that is given the choice the one they would select, is determined, amongst other things, by size, thermal insulation (amount of fur) and resting metabolic rate. For these reasons the 'comfortable' temperature will be higher in newborn than in adults. Newborns can withstand prolonged hypothermia, just as they can survive prolonged asphyxia and trauma, much better than adults. Nevertheless, hypothermia is a common cause of death. Parents, usually mothers, often go to considerable lengths to minimize the effects of the cold environment. Thus, they provide nests in which a litter can huddle (e.g. rabbits) and they may adjust the time they spend huddled with their young in the nest according to ambient temperature (rats and mice in cold environments), they may go to great lengths to select a warm dark protected spot (cats) or hide their young deep in their fur (polar bears in ice caves) or continually cuddle their young so that they become a part, as it were, of the maternal body, thus negating the disadvantage that follows the body weight–surface area ratio. It is the behavioural characteristics of the parents which are paramount in keeping their young warm to ensure their survival.

However, the young also make behavioural responses within minutes of birth aimed at keeping themselves warm (D. Hull, 1982). The blind, comparatively hairless newborn rabbit senses the cold and drags itself, given a choice, to a warm spot which it will select to ±1°C (J. Hull and Hull, 1982). It is warmth that attracts the newborn and creates the huddle in a litter, and probably draws them to their mother when she visits the nest to feed (once or twice daily). Newborn rats clustered in a ball take turns to be on the outside or in the middle (Alberts, 1978). Similarly, piglets in a row continually move their positions so that the same piglet is not always at the end.

The behavioural responses of newborn human infants to overheating are limited to reducing insulation by kicking the covers off, putting hands or feet outside the bedclothes or adopting the 'sunbathing' posture which exposes the greatest possible surface area to the air (Rutter and Hull, 1979a). The inadequacy of these behavioural responses is suggested by the epidemiological associ-

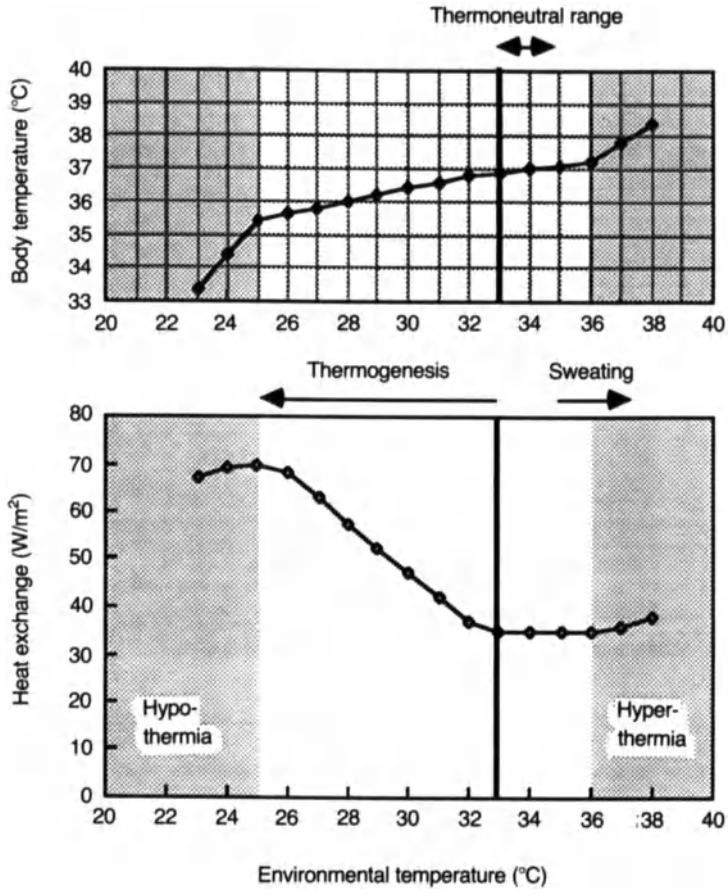

Figure 8.24 Thermoregulatory diagram. Data shown are for a naked infant at term.

ations between both sleeping prone and the amount of bedclothes and an increased risk of sudden unexpected death in infancy. The face and forehead account for up to 25% of the surface responsible for heat loss in the newborn infant, and it is thought that the prone infant cannot lose heat effectively and that overheating is a crucial step in 'cot death' (Anonymous, 1990).

It was once thought that newborn mammals were poikilothermic. Certainly some have very little capacity for thermogenesis for some days after birth (hamsters, lemmings) (Edson and Hull, 1981); others develop a considerable thermogenic capacity within the first 2 or 3 days (mice and rats) (Vinter and Hull, 1982), whilst others show a striking response within minutes of birth (guinea pigs, rabbits, pigs, lambs) (Holloway, 1984). In most newborn mammals the site of this non-shivering thermogenesis is brown adipose tissue (Nicholls, 1984; Cannon, 1985) (rabbits, guinea pigs), but in others it is a mixture of brown adipose tissue and shivering (lamb and ox), whilst the piglet appears to depend on shivering alone (G. Alexander, 1975). The human infant has a modest metabolic response to cold within hour of birth, but by 24 h cold exposure can double the metabolic rate (Ricquier, 1984). However, it is rabbits, pigs and lambs that have been shown to have impressive metabolic responses to

cold exposure. They can often treble their minimal rate within minutes of cold exposure, demonstrating in another way their considerable cardiorespiratory reserve (Hey, 1969).

Brown adipose tissue is yet another illustration of the wide variation between species (Benito, 1985). In some species it is a major organ and when fully active it can consume twice as much oxygen as the rest of the body; in others it is absent (D. Hull, 1966, 1970). But it also illustrates why the study of fetal physiology is of interest for its own sake, for the tissue in many species develops in the uterus where it has no function, to respond at birth and in the early phase of independent existence only to bow from the stage before adulthood.

8.5 CONCLUSION

This chapter is too brief to be comprehensive. Inevitably the topics which have been emphasized reflect the authors' own interest and in that sense it lacks balance. Likewise, not every assertion is supported by a reference to the relevant observations and investigations. The aim has been to provide the reader with an introduction to some of the characteristics of fetal physiology and the various ways they have been studied.

Much remains to be learnt about the fetus in mid-gestation, about the determinants of organ growth and development, on how the fetus controls its nutrient uptake and gaseous exchange and about the mechanisms and determinants of placental function.

REFERENCES

Abramovich, D.R. (1970) Fetal factors influencing amniotic fluid volume and composition of liquor amnii. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **77**, 865–877.

Abrams, R.M. (1978) Thermal physiology of the fetus, in *Temperature Regulation and Energy Metabolism in the Newborn*, (ed. J.C. Sinclair), Grune & Stratton, New York.

Ackerman, G.A. (1967) The lymphocyte: its morphology and embryological origin, in *The Lymphocyte in Immunology and Haemopoiesis*, (ed. J.M. Yoffey), Edward Arnold, London, pp. 11–30.

Adamson, T., Brodecky, V., Lambert, T., Maloney, J., Ritchie, B. and Walker, A. (1973) The production and composition of lung liquid in the in-utero fetal lamb, in *Fetal and Neonatal Physiology* (eds K. Comline, K. Cross, G.S. Dawes and P.W. Nathanielsz), Bycroft Centenary Symposium, Cambridge University Press, Cambridge, UK.

Alberts, J.R. (1978) Huddling by rat pups: Group behavioural mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology*, **92**, 231–245.

Alcorn, D., Adamson, T., Lambert, T., Maloney, J., Ritchie, B. and Robinson, P. (1977) Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. *Journal of Anatomy*, **123**, 649–660.

Al-Dahhan, J., Haycock, G.B., Chantler, C. and Stimmler, L. (1983a) Sodium homeostasis in terms and preterm neonates. 1. Renal aspects. *Archives of Disease in Childhood*, **58**, 335–342.

Al-Dahhan, J., Haycock, G.B., Chantler, C. and Stimmler, J. (1983b) Sodium homeostasis in term and preterm neonates. II. Gastrointestinal aspects. *Archives of Disease in Childhood*, **58**, 343–345.

Alexander, D.P. and Nixon, D.A. (1961) The foetal kidney. *British Medical Bulletin*, **17**, 112–117.

Alexander, D.P. and Nixon, D.A. (1962) Plasma clearance of p-aminohippuric acid by the kidneys of foetal, neonatal and adult sheep. *Nature*, **194**, 483–484.

Alexander, D.P. and Nixon, D.A. (1963) Reabsorption of glucose, fructose and mesoinositol by the foetal and postnatal sheep kidney. *Journal of Physiology*, **167**, 480–486.

Alexander, D.P., Nixon, D.A., Widdas, W.F. and Wohlzogen, F.X. (1958) Gestational variations in the composition of foetal fluids and foetal urine in the sheep. *Journal of Physiology*, **140**, 1–13.

Alexander, G. (1975) Body temperature control on mammalian young. *British Medical Bulletin*, **31**, 62–68.

Alpan, G. and Glyman, R. I. (1990). The effect of methylprednisolone on hypoxic pulmonary vasoconstriction in the newborn late-gestation lamb. *Pediatric Research*, **27(2)**, 133–136.

Alt, J.M., Colenbrander, R., Forsling, M.L. and Macdonald, A.A. (1984) Perinatal development of tubular function in the pig. *Quarterly Journal of Experimental Physiology*, **609**, 693–702.

Andersen, H., Bierring, F., Matthiessen, M., Egeberg, J. and Bro-Rasmussen, F. (1964) On the nature of meconium corpuscles in human foetal intestinal epithelium. *Acta Pathologica et Microbiologica Scandinavica*, **61**, 377–393.

Anderson, R., Benolken, R., Dudley, P., Landis, D. and Wheeler, T. (1974). Polyunsaturated fatty acids of photoreceptor membranes. *Experimental Eye Research*, **18**, 205–213.

Andrews, N.P., Broughton-Pipkin, F. and Heptinstall, S. (1985) Blood platelet behaviour in mothers and neonates. *Thrombosis and Haemostasis*, **53**, 428–432.

Anonymous (1987) Erythropoietin. *Lancet*, **i**, 781–782.

Anonymous (1990) Prone, hot and dead. *Lancet*, **ii**, 1104.

Anthony, M., Goodall, S., Pappuli, M. and Levene, M. (1992) Measurement of plasma volume in neonates. *Archives of Disease in Childhood*, **67**, 36–40.

Aoki, A. (1966) Development of the human renal glomerulus. 1. Differentiation of the filtering membrane. *Anatomical Record*, **155**, 339–352.

Aperia, A. and Elinder, G. (1981) Distal tubular sodium reabsorption in the developing rat kidney. *American Journal of Physiology*, **240**, F487–F491.

Aperia, A. and Zetterstrom, R. (1982) Renal control of fluid homeostasis in the newborn infant. *Clinics in Perinatology*, **9**, 523–533.

Aperia, A., Larsson, L. and Zetterstrom, R. (1981) Hormonal induction of Na-K-ATPase in developing proximal tubular cells. *American Journal of Physiology*, **241**, 356–360.

Aperia, A., Herin, P., Lundin, S., Melin, P. and Zetterstrom, R. (1984) Regulation of renal water excretion in newborn full-term infants. *Acta Paediatrica Scandinavica*, **73**, 717–721.

Apgar, V. (1953) A proposal for a new method of evaluation of the newborn infant. *Current Research in Anesthetics and Analgesia*, **32**, 260–267.

Arey, L.B. (1974) *Developmental anatomy*, W.B. Saunders, Philadelphia.

Atkinson, S. (1992) Feeding the normal term infant, in *Effective Care of the Newborn Infant*, (eds J. Sinclair and M. Bracken), Oxford University Press, Oxford, pp. 73–93.

Attal, J. (1969) Levels of testosterone, androstenedione, estrone and estradiol 17β in the testes of fetal sheep. *Endocrinology*, **85**, 280–289.

Avery, M.E. and Mead, J. (1959) Surface properties in relation to atelectasis and hyaline membrane disease. *American Journal of Diseases in Children*, **97**, 517–523.

Bailey, D.J., Bradbury, M.W.B. and France, V. (1979) Cation transport across the guinea-pig placenta perfused in situ. *Journal of Physiology*, **207**, 45–56.

Bailey, E. and Lockwood, E. (1973) Some aspects of fatty acid oxidation and ketone body formation and utilization during development of the rat. *Enzyme*, **15**, 239–253.

Bailie, M.D., Derkx, F.H.M. and Schalekamp, A.D.H. (1980) Release of active and inactive renin by the pig kidney during development. *Developmental Pharmacology and Therapeutics*, **1**, 47–57.

Banks-Schlegel, S.P. (1982) Keratin alterations during embryonic epidermal differentiation: a presage of adult epidermal maturation. *Journal of Cell Biology*, **93**, 551–559.

Barcroft, J. (1946) *Researches on Prenatal Life*, Oxford Blackwell Scientific Publications.

Bard, H., Fouron, J.-C., Robillard, J.E., Cornet, A. and Soukini, M.A. (1978) Red cell oxygen affinity in fetal sheep: role of 2, 3–DPG and adult haemoglobin. *Journal of Applied Physiology*, **45**, 7–10.

Barker, D.J.P., Bull, A.R., Osmond, C. and Simmond, S.J. (1990). Fetal and placental size and risk of hypertension in adult life. *British Medical Journal*, **301**, 259–262.

Barnes, N. (1992) Endocrine disorders, in *Textbook of Neonatology*, 2nd edn (ed. N. Roberton), Churchill Livingstone, Edinburgh, pp. 799–837.

Barnes, R.J., Comline, R.S., Dobson, A., Silver, M., Burton, G.J. and Steven, D.H. (1979) On the presence of a ductus venosus in the fetal pig in late gestation. *Journal of Developmental Physiology*, **1**, 105–110.

Bassett, J. and Madill, D. (1974) Influence of prolonged glucose infusions on plasma insulin and growth hormone concentrations of foetal lambs. *Journal of Endocrinology*, **62**, 299–309.

Battaglia, F.C., Artini, P. G., D'Ambrogio, G., Galli, P. A., Segre, A. and Genazzani, A. R. (1992). Maternal hyperoxygenation in the treatment of intrauterine growth retardation. *American Journal of Obstetrics and Gynecology*, **167**, 430–435.

Battaglia, F.C. and Hay, W. (1984). Energy and

substrate requirements for fetal and placental growth and metabolism, in *Fetal Physiology and Medicine*, (eds R. Beard and P. Nathanielsz), Marcel Dekker, New York, pp. 601–628.

Battaglia, F.C. and Meschia, G. (1988) Principal substrates of fetal metabolism. *Physiological Reviews*, **58**, 499–527.

Battaglia, F.C. and Meschia, G. (1986) *An Introduction to Fetal Physiology*, Academic Press, New York.

Battaglia, F. C. and Meschia, G. (1988) Fetal Nutrition. *Annual Reviews of Nutrition*, **8**, 43–61.

Battaglia, F.C., McGaughey, H., Makowski, E.L. and Meschia, G. (1970) Postnatal changes in oxygen affinity of sheep red cells: a dual role of diphosphoglyceric acid. *American Journal of Physiology*, **219**, 217–221.

Baum, J.D. and Harris, D. (1972) Colloid osmotic pressure in erythroblastosis fetalis. *British Medical Journal*, **i**, 601–603.

Baum, J.D., Eisenberg, C., Franklin, F.A., Meschia, G. and Battaglia, F.C. (1971) Studies on colloid osmotic pressure in the fetus and newborn infant. *Biology of the Neonate*, **18**, 311–320.

Beitins, I.Z., Bayard, F., Levitsky, L., Ances, I.G., Kowarski, A. and Migeon, C.J. (1972) Plasma aldosterone concentration at delivery and during the newborn period. *Journal of Clinical Investigation*, **51**, 386–394.

Benavides, C., Perez, R., Espinoza, M., Cabello, G., Riguelme, R., Parer, J. and Llano, A. (1989). Cardiorespiratory functions in the fetal llama. *Respiration Physiology*, **75**, 327–334.

Benito, M. (1985). Contribution of brown fat to neonatal thermogenesis. *Biology of the Neonate*, **48(4)**, 245–249.

Birch, T. (1956–7) *The History of the Royal Society of London*, **1**, 180.

Biscoe, T., Purves, M. and Sampson, S. (1969) Types of nervous activity which may be recorded from the carotid sinus nerve in the sheep fetus. *Journal of Physiology*, **202**, 1–23.

Black, V.H. and Christensen, A.K. (1969) Differentiation of interstitial cells and Sertoli cells in fetal guinea pig testes. *American Journal of Anatomy*, **124**, 211–238.

Bland, R.D. (1986) *Lung Fluid Balance Before and After Birth in Respiratory Control and Lung Development in the Fetus and Newborn*, (eds B.M. Johnson and P.D. Gluckman), Perinatal Press, New York.

Blott, M., Greenough, A., Nicolaides, K., Moscoso, G., Gibb, D. and Campbell, S. (1987) Fetal breathing movements as a predictor of favourable pregnancy outcome after oligohydramnios due to membrane rupture in the second trimester. *Lancet*, **ii**, 129–131.

Boyd, R.D.H., Haworth, C., Stacey, T.E. and Ward, R.H.T. (1976) Permeability of the sheep placenta to unmetabolised polar non-electrolytes. *Journal of Physiology*, **256**, 617–634.

Boyden, E. (1977) Development and growth of the airways, in *Development of the Lung*, (ed. W. Hodson), Marcel Dekker, New York, pp 3–35.

Boyland, J.W., Colbourn, E.P. and McCance, R.A. (1958) Renal function in the foetal and newborn guinea pig. *Journal of Physiology*, **141**, 323–331.

Bradley, R.M. and Mistretta, C.M. (1973) The gustatory sense in foetal sheep during the last third of gestation. *Journal of Physiology*, **231**, 271–282.

Brandes, R., Arad, R. and Barash, V. (1987) Cytosolic fatty acid binding proteins in rat placenta. *Placenta*, **8**, 347–350.

Bremer, J.L. (1916) The inter-relations of the mesonephros, kidney and placenta in different classes of animals. *American Journal of Anatomy*, **19**, 179–210.

Brenner, R. (1984) Effect of unsaturated acids on membrane structure and enzyme kinetics. *Progress in Lipid Research*, **23**, 69–96.

Bressler, M. and Munger, B.L. (1983) Embryonic maturation of sensory terminals of primate facial hairs. *Journal of Investigative Dermatology*, **80**, 245–260.

Brod, J. (1973) *The Kidney*, Butterworths, London.

Brody, B., Kinney, H., Kloman, A. and Gilles, F. (1987). Sequence of central nervous system myelination in human infancy. *Journal of Neuropathology and Experimental Neurology*, **46**, 283–301.

Broughton-Pipkin, F. (1973) Vasoactive hormones in immature mammals. *D Phil Thesis*, University of Oxford.

Broughton-Pipkin, F. and Symonds, E.M. (1984) Renin–angiotensin system in early life, in *Fetal Medicine and Physiology*, (eds R.W. Beard and P. Nathanielsz), Marcel Dekker, New York, pp. 459–480.

Broughton-Pipkin, F., Ousey, J., Wallace, C.P. and Rossdale, P.D. (1984) The effect of salt and water loss on the renin–angiotensin aldosterone system in the newborn foal. *Equine Veterinary Journal*, **16**, 292–297.

Brunette, M.G. (1989) Effects of parathyroid hor-

mone on PO_4 transport through the human placenta microvilli. *Pediatric Research*, **25**, 19–26.

Cain, H. and Krause, B. (1971) Der Juxtaglomerulare Apparat der Rattenniere im Verlauf von Wachstum, Reifung und Alterung. *Virchows Archiv*, **9**, 164–179.

Calcagno, P.L. and Rubin, M.I. (1963) Renal extraction of p-aminohippurate in infants and children. *Journal of Clinical Investigation*, **42**, 1632–1639.

Canning, J. and Boyd, R. (1984) Mineral and water exchange between mother and fetus, in *Fetal Physiology and Medicine*, (eds R. Beard and P. Nathanielsz), Marcel Dekker, New York, pp 481–509.

Cannon, B. (1985) The biochemistry of an inefficient tissue: brown adipose tissue. *Essays in Biochemistry*, **20**, 110–164.

Care, A.D. and Ross, R. (1984) Fetal calcium homeostasis. *Journal of Developmental Physiology*, **6**, 59–66.

Carlson, S., Rhodes, P. and Ferguson, M. (1986) Docosohexanoic acid status of preterm infants at birth and following feeding with human milk or formulas. *American Journal of Clinical Nutrition*, **44**, 798–804.

Cartlidge, P.H.T. and Rutter, N. (1986) Serum albumin concentrations and oedema in the newborn. *Archives of Disease in Childhood*, **61**, 657–660.

Cartlidge, P.H.T. and Rutter, N. (1987) Percutaneous respiration in the newborn infant. *Biology of the Neonate*, **54**, 68–72.

Catt, K.J., Dufau, M.L., Neaves, W.B., Walsh, P.C. and Wilson, J.D. (1975) LH-hCG receptors and testosterone content during differentiation of the testis in the rabbit embryo. *Endocrinology*, **67**, 1157–1165.

Chamberlain, G.V.P. and Wilkinson, A.E. (1979) *Placental Transfer*, Pitman Medical, UK.

Charlton, V.E., Reis, B.L. and Lofgren, D.J. (1979a) Consumption of carbohydrates, amino acids and oxygen across the intestinal circulation in the fetal sheep. *Journal of Developmental Physiology*, **1**, 329–336.

Charlton Char, V. and Rudolph, A.M. (1979b) Digestion and absorption of carbohydrates by the fetal lamb *in utero*. *Pediatric Research*, **13**, 1018–1023.

Cheung, C.Y. and Brace, R.A. (1988) Fetal hypoxia elevates plasma atrial natriuretic factor concentration. *American Journal of Obstetrics and Gynecology*, **159**, 1263–1268.

Cheung, C.Y. and Brace, R.A. (1989). Haemorrhage induced reduction of plasma atrial natriuretic factor in the ovine fetus. *International Union of Physiological Societies, Satellite Symposium*, Stockholm, (Abstract A3), 89.

Chevalier, R.L. (1982) Glomerular number and perfusion during normal and compensatory renal growth in the guinea pig. *Pediatric Research*, **16**, 436–440.

Chez, R.A., Smith, F.G. and Hutchinson, D.L. (1964) Renal function in the intrauterine primate fetus. *American Journal of Obstetrics and Gynecology*, **90**, 128–131.

Chez, R.A., Mintz, D.H. and Hutchinson, D.L. (1974) Gastric glucose tolerance in the third trimester monkey conceptus. *American Journal of Obstetrics and Gynecology*, **120**, 553–554.

Clements, J.A. (1977) Functions of the alveolar lining layer. *American Review of Respiratory Disease*, **115**, 67–71.

Clements, J.A., Reyes, F.I., Winter, J.S.D. and Faiman, C. (1976) Studies on human sexual development. III. Fetal pituitary and serum amniotic fluid concentrations of LH, CH and FSH. *Journal of Clinical Endocrinology and Metabolism*, **42**, 9–19.

Clements, M.R. (1988) Vitamin D supply to the rat fetus and neonate. *Journal of Clinical Investigation*, **81**, 7768–7773.

Cohen, M. and Carson, B. (1985) Respiratory morbidity benefit of awaiting onset of labour after elective Caesarean section. *Obstetrics and Gynecology*, **65**, 818–824.

Cole, B.R., Brocklebank, J.T., Capps, R.G., Murray, B.N. and Robson, A.M. (1972) Maturation of p-aminohippuric acid transport in the developing rabbit kidney: interrelationships of the individual components. *Pediatric Research*, **12**, 992–997.

Cole, R.J. and Paul, J. (1966) The effects of erythropoietin on haem synthesis in mouse yolk sac and cultured foetal liver cells. *Journal of Embryology and Experimental Morphology*, **15**, 245–260.

Collins, M., Moessinger, A., Kleinerman, J., James, L. and Blanc, W. (1986) Morphometry of hypoplastic fetal guinea pig lungs following amniotic fluid leak. *Pediatric Research*, **20**, 955–960.

Comline, R. S. and Silver, M. (1975). Placental transfer of blood gases. *British Medical Bulletin*, **31**, 25–31.

Comroe, J. (1977) *Retrospectroscope*, Von Gehr Press, Menlo Park, California.

Coulthard, M. (1985) Maturation of glomerular filtration in preterm and mature babies. *Early Human Development*, **11**, 281–292.

Court, D.J., Block, B.S.B. and Llanos, A.J. (1984) Effects of β-adrenergic blockade on blood flow distribution during hypoxaemia in fetal sheep. *Journal of Developmental Physiology*, **6**, 349–358.

Cross, K.W. (1979) La chaleur animale and the infant brain. *Journal of Physiology*, **294**, 1–21.

Crowley, P., Chalmers, I. and Keirse, M. (1990) The effects of corticosteroid administration before preterm delivery: an overview of the evidence from controlled trials. *British Journal of Obstetrics and Gynaecology*, **97**, 11–25.

Dancis, J., Braverman, N. and Lind, J. (1957) Plasma protein synthesis in the human fetus and placenta. *Journal of Clinical Investigation*, **36**, 398–404.

Dancis, J., Jansen, V. and Levitz, M. (1980) Placental transfer of steroids: Effect of binding to serum albumin and to placenta. *American Journal of Physiology*, **238**, 208.

Davidson, D. and Eldemerdash, A. (1990) Endothelium-derived relaxing factor: presence in pulmonary and systemic arteries of the newborn guinea pig. *Pediatric Research*, **17**, 128–132.

Davies, J. (1951) Nephric development in the sheep with reference to the problem of the ruminant pronephros. *Journal of Anatomy*, **85**, 6–11.

Davies, J. (1952) Correlated anatomical and histochemical studies on the mesonephros and placenta of the sheep. *American Journal of Anatomy*, **91**, 263–300.

Dawes, G.S. (1968) *Fetal and Neonatal Physiology*, Year Book Medical Publishers, Chicago.

Dawes, G.S. (1986) The rediscovery of fetal breathing movements and its consequences, in *Respiratory Control and Lung Development in the Fetus and Newborn*, (eds B.M. Johnston and P.D. Gluckman), Perinatology Press, New York.

Dean, R.F.A. and McCance, R.A. (1949) The renal response of infants and adults to the administration of hypertonic solutions of sodium chloride and urea. *Journal of Physiology*, **109**, 81–97.

Debnam, E.S., Epstein, O., Srai, S.K.S. (1987) Developmental changes in the autographic localisation of iron uptake by the guinea pig duodenum. *Journal of Physiology*, **394**, 164.

Delivoria-Papadopoulos, M., Martens, R.J., Forster, R.E. and Oski, F.A. (1974) Postnatal changes in oxygen–hemoglobin affinity and erythrocyte 2, 3–diphosphoglycerate in piglets. *Pediatric Research*, **8**, 84–66.

De Prins, F., Hill, D.J., Milner, R.D.G. and Van Assche A. (1988) Effect of maternal hyperalimentation on intrauterine growth retardation. *Archives of Disease in Childhood*, **63**, 733–736.

Demers, L.M., Gabbe, S.G., Villee, C.A. and Greep, R.O. (1972) The effect of insulin on human placental glycogenesis. *Endocrinology*, **91**, 270.

Dennis, J., Johnson, A., Mutch, L., Yudkin, P. and Johnson, P. (1989). Acid-base status at birth and neurodevelopmental outcome at four and one-half years. *American Journal of Obstetrics and Gynecology*, **161**, 213–220.

Deren, J.S. (1971) Development of structure and function in the fetal and newborn stomach. *American Journal of Clinical Nutrition*, **24**, 144–159.

Dewitt, O., Dibba, B. and Prentice, A. (1990). Breast-milk amylase activity in English and Gambian mothers: effects of prolonged lactation, maternal parity, and individual variations. *Pediatric Research*, **28**, 502–506.

Dhiab, A.L., Naimi, A.A.H. and Bearn, J.G. (1981) The juxtaglomerular apparatus and glomerular granular cells in fetuses and neonates of the rat. *Journal of Anatomy*, **133**, 607–623.

Dobbing, J. and Sands, J. (1979) Comparative aspects of the brain growth spurt. *Early Human Development*, **3**, 79–83.

Dobrovik-Jenik, D., Ozegovic, B. and Milkovic, S. (1984) Postnatal development of rat kidney plasma membrane Na^+/K^+-ATPase. *Biology of the Neonate*, **46**, 115–121.

Duee, P.H., Pegorier, J.P., El Manoubi, L., Herbin, C., Kohl, C. and Girard, J. (1985) Hepatic triglyceride hydrolysis and development of ketogenesis in rabbits. *American Journal of Physiology*, **249**, 478–484.

East, J.M., Chepenik, K.P. and Waite, B.M. (1975) Phospholipase A activities in rat placentas of 14 days gestation. *Biochimie Biophysica Acta*, **388**, 106–112.

Eckberg, D. and Sleight, P. (1992) *The Human Baroreflex in Health and Disease*, Oxford Medical Publications, Oxford.

Edelstone, D. and Rudolph, A. (1979) Preferential streaming of the ductus venosus blood to the brain and heart in fetal lambs. *American Journal of Physiology*, **237**, H724.

Edson, J.L. and Hull, D. (1981) The development of cold induced thermogenesis in hamsters. *Journal of Developmental Physiology*, **6**, 387–396.

Edson, J.L., Hudson, D.G. and Hull, D. (1975) Evidence for increased fatty acid transfer across the placenta during a maternal fast in rabbits. *Biology of the Neonate*, **7**, 50–56.

Eguchi, Y. and Morikawa, Y. (1968) Changes in pituitary gonadal interrelations during perinatal days in the rat. *Anatomical Record*, **161**, 163–170.

Elphick, M.C. and Hull, D. (1984) Transfer of fatty acids across the cat placenta. *Journal of Developmental Physiology*, **6**, 517–525.

Elphick, M.C., Edson, J.L., Lawlor, J.P. and Hull, D. (1978a) Source of fetal-stored lipids during maternal starvation in rabbits. *Biology of the Neonate*, **34**, 146–149.

Elphick, M.C., Harrison, A.T., Lawlor, J.P. and Hull, D. (1978b) Cord blood hypertriglyceridaemia as an index of fetal stress: use of a simple screening test and results of further biochemical analysis. *British Journal of Obstetrics and Gynaecology*, **85**, 303–310.

Elphick, M.C., Hull, D. and Broughton-Pipkin, F. (1979) The transfer of fatty acids across the sheep placenta. *Journal of Developmental Physiology*, **1**, 31–45.

Elphick, M.C., Flecknell, P., Hull, D. and McFadyen, I.R. (1980) Plasma free fatty acid umbilical venous–arterial concentration differences and placental transfer of [^{14}C] palmitic acid in pigs. *Journal of Developmental Physiology*, **2**, 347–356.

Emery, J.L. (1956) The distribution of haemopoietic foci in the infantile human liver. *Journal of Anatomy*, **90**, 293–297.

Euler, A.R., Byrne, W.J., Cousins, L.M., Ament, M.E., Leake, R.D. and Walsh, J.H. (1977) Increased serum gastrin concentration and gastric acid hyposecretion in the immediate newborn period. *Gastroenterology*, **72**, 1271–1273.

Euler, A.R., Byrne, W.J. and Meis, P.J. (1979) Basal and pentagastrin stimulated acid secretion in newborn human infants. *Pediatric Research*, **13**, 36–37.

Evans, N.J. and Rutter, N. (1986a) Development of the epidermis in the newborn. *Biology of the Neonate*, **49**, 78–80.

Evans, N.J. and Rutter, N. (1986b) Percutaneous respiration in the newborn infant. *Journal of Pediatrics*, **108**, 282–286.

Faber, J.J. and Hart, F.M. (1966) The rabbit placenta as an organ of diffusional exchange. Comparison with other species by dimensional analysis. *Circulation Research*, **19**, 816–833.

Faber, J.J. and Thornburg, K.L. (1983) *Placental Physiology*, Raven Press, New York.

Farbman, A.I. (1965) Electron microscope study of the developing taste bud in rat fungiform papilla. *Developmental Biology*, **11**, 110–135.

Fetterman, G.H., Shuplock, N.A., Philipp, F.J. and Gregg, H.S. (1965) The growth and maturation of human glomeruli and proximal convolutions from term to adulthood: studies by microdissection. *Pediatrics*, **35**, 601–619.

Fisk, N.M. and Rodeck, C.H. (1992) Antenatal diagnosis and fetal medicine, in *Textbook of neonatology*, 2nd edn (ed. N.R.C. Roberton), Churchill Livingstone, Edinburgh, pp. 121–150.

Flexner, L.B. and Gellhorn, A. (1942) Comparative physiology of placental transfer. *American Journal of Obstetrics and Gynecology*, **43**, 965–974.

Forestier, F. (1987) Some aspects of fetal biology. *Fetal Therapy*, **2**, 181–187.

Forestier, F., Daffos, F., Galacteros, F., Bardakjian, J., Rainaut, M. and Beuzard, Y. (1986) Hematological values of 163 normal fetuses between 18 and 30 weeks gestation. *Pediatric Research*, **20**, 342–346.

Forestier, F., Daffos, F., Rainaut, M., Bruneau, M. and Trivin, F. (1987) Blood chemistry of normal human fetuses at midtrimester of pregnancy. *Pediatric Research*, **21**, 579–583.

Forte, J.G., Forte, T.M., Machen, T.E., Cook, B. and Nalbandov, A.V. (1975) Histamine-stimulated hydrogen ion secretion by *in vitro* piglet gastric mucosa. *Journal of Physiology*, **244**, 15–31.

Foster, D.L., Cruz, T.A.C. and Jackson, G.L. (1972) Regulation of luteinizing hormone in the fetal and neonatal lamb. III. Release of LH by the pituitary *in vivo* in response to crude ovine hypothalamic extract or purified porcine gonadotrophin releasing factor. *Endocrinology*, **90**, 673–683.

Fowden, A.L. (1980) Effects of adrenalin and amino acids on the release of insulin in the sheep fetus. *Journal of Endocrinology*, **87**, 113–121.

Fowden, A. (1989) The role of insulin in prenatal growth. *Journal of Developmental Physiology*, **12**, 173–182.

Fowden, A.L., Comline, R.S. and Silver, M. (1984) Insulin secretion and carbohydrate metabolism during pregnancy in the mare. *Equine Veterinary Journal*, **16**, 239–246.

Fowden, A.L., Coulson, R.L. and Silver, M. (1990) Endocrine regulation of tissue glucose–6–

phosphatase activity in the fetal sheep during late gestation. *Endocrinology*, **126**, 2823–2830.

Freemark, M. (1986) Epidermal growth factor stimulates glycogen synthesis in fetal rat hepatocytes: comparison with the glycogenic effects of insulin-like growth factor I and insulin. *Endocrinology*, **119**, 522–526.

Friis, C. (1979) Postnatal development of renal function in piglets: glomerular filtration rate, clearance of PAH and PAH extraction. *Biology of the Neonate*, **35**, 180–187.

Friis, C. (1980) Postnatal development of the pig kidney: ultrastructure of the glomerulus and the proximal tubule. *Journal of Anatomy*, **130**, 513–526.

Fumia, F.D., Edelstone, D.I. and Holzman, I.R. (1984) Blood flow and oxygen delivery to fetal organs as functions of fetal hematocrit. *American Journal of Obstetrics and Gynecology*, **150**, 274–282.

Gartner, L.M. (1981) Disorders of bilirubin metabolism, in *Haematology of infancy and childhood*, 2nd edn, (eds D.G. Nathan and F.A. Oski), W.B. Saunders, Philadelphia, pp. 86–118.

Garzon, B., Ducroc, R. and Geloso, J.-P. (1981) Ontogenesis of gastric acid secretion in the fetal rat. *Pediatric Research*, **1**, 921–925.

Garzon, B., Ducroc, R. and Geloso, J.-P. (1982) Ontogenesis of gastric response to agonists and antagonists of acid secretion in fetal rat. *Journal of Developmental Physiology*, **4**, 195–205.

Gennser, G., Liedholm, P. and Thorell, J. (1976) Pituitary hormone levels in plasma of the human fetus after administration of LRH. *Journal of Clinical Endocrinology and Metabolism*, **43**, 470–473.

Gespach, C., Dupont, C. and Rosselin, G. (1981) Action of histamine and vasoactive intestinal peptide (VIP) on cyclic AMP in gastric glands isolated from human fetal stomach. *Experientia*, **37**, 866–867.

Gilbert, G. (1993) Chickenpox during pregnancy. *British Medical Journal*, **306**, 1079–1080.

Gilbert, M. (1977) Origin and metabolic fate of plasma glycerol in rat and rabbit fetus. *Pediatric Research*, **11**, 95–99.

Girard, J. (1986) Gluconeogenesis in late fetal and early neonatal life. *Biology of the Neonate*, **50**, 237–258.

Girard, J., Pintado, E. and Ferre, P. (1979) Fuel metabolism in the mammalian fetus. *Annals de Biologie Animale, Biochimie et Biophysique*, **19**, 181–197.

Giry, J. and Delost, P. (1974) Evolution de l'aldosteronemie du foetus et du nouveau-ne de cobaye au cours de la periode perinatale. *Comptes Rendus de L'Academie des Sciences de Paris*, **279**, 2087–2090.

Gitlin, D., Kumate, J., Morales, C., Noriega, L. and Arevalo, N. (1972) The turnover of amniotic fluid protein in the human conceptus. *American Journal of Obstetrics and Gynecology*, **113**, 632–645.

Glader, B.E. and Buchanan, G.R. (1976) The bleeding neonate. *Pediatrics*, **58**, 548–555.

Gleiss, J. and Stuttgen, G. (1970) Morphological and functional development of the skin, in *Physiology of the Perinatal Period*, Vol. 2, (ed. U. Stave), Appleton Century Crofts, New York.

Gluckman, P. and Liggins, G. (1984). Regulation of fetal growth, in *Fetal Physiology and Medicine*, (eds R. Beard and P. Nathanielsz), Marcel Dekker, New York, pp. 511–557.

Goldsmith, D.I., Drukker, A., Blaufox, M.D., Edelmann, C.M. and Spitzer, A. (1979) Hemodynamic and excretory responses of the neonatal canine kidney to acute volume expansion. *American Journal of Physiology*, **237**, F392–F397.

Gomez, R.A., Meernik, J.G., Kuehl, W.D. and Robillard, J.E. (1984) Developmental aspects of the renal response to haemorrhage during foetal life. *Pediatric Research*, **18**, 40–46.

Goncharevskaya, O.A. and Dlouha, H. (1975) The development of various generations of nephrons during postnatal ontogenesis in the rat. *Anatomical Record*, **182**, 367–376.

Grand, R.J., Watkins, J.B. and Torti, F.M. (1976) Development of the human gastrointestinal tract. *Gastroenterology*, **70**, 790–810.

Green, M.R. and Couchman, J.R. (1984) Distribution of epidermal growth factor receptors in rat tissues during embryonic skin development, hair formation and the adult hair growth cycle. *Journal of Investigative Dermatology*, **83**, 1118–1123.

Greenough, A., Morley, C. and Roberton, N. (1992) Acute respiratory disease in the newborn, in *Textbook of Neonatology*, 2nd edn, (ed. N. Roberton), Churchill Livingstone, Edinburgh, pp. 385–504.

Grinsted, J. and Aagesen, L. (1984) Mesonephric excretory function related to its influence on differentiation of fetal gonads. *Anatomical Record*, **210**, 551–556.

Gruenwald, P. (1952) Development of the excretory system. *Annals of the New York Academy of Sciences*, **55**, 142–146.

Gruskin, A.B., Edelmann, G.M. and Yuan, S. (1970) Matural changes in renal blood flow in piglets. *Pediatric Research*, **4**, 7–13.

Guignard, J.-P. (1982) Renal function in the newborn infant. *Pediatric Clinics of North America*, **29**, 777–790.

Gustafsson, J., Andersson, S. and Sjovall, J. (1985) Bile acid metabolism during development: metabolism of taurodeoxycholic acid in human fetal liver. *Biology of the Neonate*, **47**, 26–31.

Hallidie-Smith, K. (1984) Prostaglandin E_1 in suspected ductus-dependent cardiac malformation. *Archives of Disease in Childhood*, **59**, 1020–1026.

Hambley, H. and Mufti, G. (1990) Erythropoietin: an old friend revisited. *British Medical Journal*, **300**, 621–622.

Hammerlund, K., Hellsing, K., Sedin, G. and Stromberg, B. (1984) Effects of human albumin infusion on transepidermal water loss in newborn infants. *Biology of the Neonate*, **45**, 112–118.

Hanson, M. (1993) *Fetus and Neonate (Physiology and Clinical Applications)*, Cambridge University Press, Cambridge.

Hardy, K. (1980) Bile acid metabolism in fetal sheep: Perinatal changes in bile acid pool. *Journal of Physiology*, **309**, 1–7.

Hargrave, B., Roman, C., Morville, P. and Heymann, M. (1990) Pulmonary vascular effects of exogenous atrial natriuretic peptide in sheep fetuses. *Pediatric Research*, **27**, 140–143.

Harpin, V.A. and Rutter, N. (1982) Development of emotional sweating in the newborn infant. *Archives of Disease in Childhood*, **57**, 691–695.

Harpin, V.A. and Rutter, N. (1983) Barrier properties of the newborn infant's skin. *Journal of Pediatrics*, **102**, 419–425.

Harris, G.W. and Levine, S. (1965) Sexual differentiation of the brain and its experimental control. *Journal of Physiology (London)*, **181**, 739–740.

Harris, P.J., Thomas, D. and Morgan, T. O. (1987) Atrial natriuretic peptide inhibits angiotensin–stimulated proximal tubular sodium and water reabsorption. *Nature*, **326**, 697–698.

Harrison, M.R., Jeston, J.A. and Ross, N.A. (1980) Correction of congenital diaphragmatic hernia *in utero*. I. The model: Intrathoracic balloon produces fetal pulmonary hypoplasia. *Surgery*, **88**, 174–182.

Harvey, J.W., Asquith, R.L. and McNulty, P.K. (1984) Haematology of foals up to one year old. *Equine Veterinary Journal*, **16**, 347–353.

Harvey, W. (1651) *Excercitationes de Generationes Animalium*, Cambridge, UK.

Haugel, A., Leturque, A., Gilbert, M., Kande, J. and Girard, J. (1988) Glucose utilization by the placental and fetal tissues in fed and fasted pregnant rabbits. *Pediatric Research*, **23**, 480–483.

Hay, W. (1979) Fetal glucose metabolism. *Seminars in Perinatology*, **3**, 157–176.

Hayward, A.F. (1967) The ultrastructure of developing gastric parietal cells in the foetal rabbit. *Journal of Anatomy*, **101**, 69–81.

Helander, H.F. (1969) Ultrastructure and function of gastric parietal cells in the rat during development. *Gastroenterology*, **56**, 35–52.

Helms, P. (1992). Growth of the lung. *Growth Matters*, **1**, 2–3.

Hendrickse, W., Stammers, J. P. and Hull, D. (1985) The transfer of free fatty acids across the human placenta. *British Journal of Obstetrics and Gynaecology*, **92**, 945–952.

Hensinger, R.N. and Jones, E.T. (1992) Orthopaedic problems in the newborn, in *Textbook of Neonatology*, (ed. N.R.C. Roberton), Churchill Livingstone, Edinburgh, pp. 889–914.

Herbst, J.J. (1989) Development of suck and swallow, in *Human Gastrointestinal Development*, (ed. E. Lebenthal), Raven Press, New York, pp. 229–239.

Herbst, J.J. and Sunshine, P. (1969) Postnatal development of the small intestine of the rat. *Pediatric Research*, **33**, 251–254.

Hershfield, M.S. and Nemeth, A.M. (1968) Placental transport of free palmitic and linoleic acids in the guinea-pig. *Journal of Lipid Research*, **9**, 460–468.

Hey, E. N. (1969) Environmental temperature and oxygen consumption in newborn babies. *Journal of Physiology*, **200**, 589–603.

Heymann, M., Rudolph, A. and Silverman, N. (1976) Closure of the ductus arteriosus in premature infants by inhibition of prostaglandin synthesis. *New England Journal of Medicine*, **295**, 530–533.

Hill, K.J. (1956) Gastric development and antibody transference in the lamb with some observations on the rat and guinea pig. *Quarterly Journal of Experimental Physiology*, **41**, 421–432.

Hislop, A. and Reid, L. (1981) The growth and development of the respiratory system, in *Scientific Foundation of Paediatrics*, (eds J.A. Davis and J. Dobbin), Wm Neinemann, London, pp. 390–432.

Hodson, W. (1992) Normal and abnormal structural development of the lung, in *Fetal and*

Neonatal Physiology, (eds R. Polin and W. Fox), W B Saunders, Philadelphia, pp. 771–782.

Hofman, M.A. (1983) Evolution of brain size in neonatal and adult placental mammals: a theoretical approach. *Journal of Theoretical Biology*, **105**, 317–332.

Holbrook, K.A. and Odland, G.F. (1980) Regional development of the human epidermis in the first trimester embryo and the second trimester fetus (ages related to the timing of amniocentesis and fetal biopsy). *Journal of Investigative Dermatology*, **80**, 161–168.

Holliman, R.E. (1992) Toxoplasma gondii, in *Congenital, Perinatal and Neonatal Infections*, (eds A. Greenough, O. Osborne and S. Sutherland), Churchill Livingstone, Edinburgh, pp. 209–221.

Holloway, B.R. (1984) Post-natal development of brown adipose tissue in guinea pig – effect of environmental temperature. *International Journal of Obesity*, **8**, 295–303.

Holter, P.H., Sanengen, T., Halvorsen, S. and Refsum, H.E. (1987) Regulation of erythropoiesis in suckling rabbits with and without postnatal anemia: partial suppression of production/release of erythropoiesis stimulating factor(s) by ion supplements. *Pediatric Research*, **21**, 1–4.

Holzgreve, W., Fisk, A.S., Goldsmith, P.C. and Goldbus, M.S. (1985) Histochemical demonstration of ornithine transcarbamylase activity in fetal liver. *Biology of the Neonate*, **47**, 339–342.

Hooper, S.B. and Harding, R. (1990) Changes in lung liquid dynamics induced by prolonged fetal hypoxemia. *Journal of Applied Physiology*, **69**, 127–135.

Horster, M. and Larsson, L. (1976) Mechanism of fluid adsorption during proximal tubule development. *Kidney International*, **10**, 348–363.

Horster, M. and Lewy, J.E. (1970) Filtration fraction and extraction of PAH during the neonatal period in the rat. *American Journal of Physiology*, **219**, 1061–1065.

Horster, M. and Valtin, H. (1971) Postnatal development of renal function: micropuncture and clearance studies in the dog. *Journal of Clinical Investigation*, **50**, 779–795.

Horvath, A., Butkus, A., MacIsaac, R.J., Pontefract, L. and Wintour, E.M. (1988) Effect of hormone-induced premature parturition on haemoglobin switching in sheep. *Pediatric Research*, **24**, 688–692.

Houston, I.B. and Zeis, P.M. (1976) Intrauterine renal function and the amniotic fluid. *Journal of Physiology*, **257**, 20–21.

Huhtaniemi, I.T., Korenbrot, C.C., Seron-Ferre, M., Foster, D.B., Parer, J.T. and Jaffe, R.B. (1977) Stimulation of testosterone production *in vivo* and *in vitro* in the male rhesus monkey fetus in late gestation. *Endocrinology*, **100**, 839–844.

Huhtaniemi, L.T., Nozu, K., Warren, D.W., Dufau, M.L. and Catt, K.J. (1982) Acquisition of regulatory mechanisms for gonadotropin receptors and steroidogenesis in the maturing rat testis. *Endocrinology*, **111**, 1711–1720.

Hull, D. (1966) The structure and function of brown adipose tissue. *British Medical Bulletin*, **22**, 92–96.

Hull, D. (1970) Brown adipose tissue in the newborn, in *Scientific Foundations of Obstetrics and Gynaecology*, (eds E.E. Philipp, J. Barnes and M. Newton), Wm Heinemann Books, London.

Hull, D. (1976) *Nutritional Endowments at Birth in Early Nutrition and Later Development*, (ed. A.W. Wilkinson), Pitman Medical Publishing Co., Kent, UK, pp. 49–55.

Hull, D. (1982) Behavioural thermoregulation in the newborn, in *Topics in Perinatal Medicine*, (ed. B. Wharton), Pitman.

Hull, J. and Hull, D. (1982) Behavioural thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology*, **285**, 924–926.

Hutchinson, D.L., Gray, M.J., Plentl, A.A., Alvarez, H., Caldeyro-Barcia, H., Kaplan, B. and Lind, J. (1959) The role of the fetus in the water exchange of the amniotic fluid of normal and hydramniotic patients. *Journal of Clinical Investigation*, **38**, 971–980.

Hyman, A.I., Levin, D.L., Rudolph, A.M. and Heymann, M.A. (1975). Angiotensin is not the mediator of hypoxia induced pulmonary vasoconstriction in foetal lambs. *Circulation*, **52**, 132.

Hyman, P.E., Clarke, D.D., Everett, S.L., Sonne, B., Stewart, D., Harrada, T., Walsh, J.H. and Taylor, I.L. (1985) Gastric acid secretory function in pre-term infants. *Journal of Pediatrics*, **106**, 467–471.

Hytten, F.E. (1979) Water transfer, in *Placental Transfer*, (eds G.V.P. Chamberlain and A.W. Wilkinson), Pitman Medical, Kent, UK.

Hytten, F.E. and Leitch, I. (1971) *The Physiology of Human Pregnancy*, Blackwell Scientific Publications, Oxford, UK.

Iwamoto, H., Oh, W. and Rudolph, A.M. (1985)

Renal metabolism in fetal and newborn sheep. *Pediatric Research*, **19**, 641–644.

Iwamoto, H. and Rudolph, A. (1981) Role of renin–angiotensin system in response to haemorrhage in fetal sheep. *American Journal of Physiology*, **240**, H848–H854.

Jacobson, L.O., Marks, E.K. and Gaston, E.O. (1959) Studies on erythropoiesis. XII. The effect of transfusion-induced polycythemia in the mother on the fetus. *Blood*, **14**, 644–653.

Jarvis, L.G., Morgan, G., Smith, M.W. and Wooding, F.B.P. (1977) Cell replacement and changing transport function in the neonatal pig colon. *Journal of Physiology*, **273**, 717–729.

Jeffcott, I.B. (1975) The transfer of passive immunity to the foal and its relation to immune status after birth. *Journal of Reproduction and Fertility*, Suppl. 23, 727–733.

Jenness, R. (1974) Biosynthesis and composition of milk. *Journal of Investigative Dermatology*, **63**, 109–118.

Jobe, A. (1984) Fetal lung maturation and the respiratory distress syndrome, in *Fetal Physiology and Medicine*, (eds R. Beard and P. Nathanielsz), Marcel Dekker, New York, pp. 317–351.

Jones, C.R., McCabe, R., Hamilton, C.A. and Reid, J.L. (1985) Maternal and fetal platelet responses and adrenoceptor binding characteristics. *Thrombosis and Haemostasis*, **53**, 95–98.

Jones, C.T. (1982) *The Biochemical Development of the Fetus and Neonate*. Elsevier Biomedical Press, Oxford.

Jones, C.T. and Ashton, I.K. (1976) Lipid biosynthesis in liver slices of the foetal guinea pig. *Biochemical Journal*, **154**, 149–158.

Jones, R. and Roberton, N. (1986) Small-for-dates babies, are they really a problem? *Archives of Disease in Childhood*, **61**, 877–880.

Joppich, R., Haberle, D. and Weber, P. (1981) Studies in the immaturity of the ADH dependent cAMP system in conscious newborn piglets – possible impairing effects of renal prostaglandins. *Pediatric Research*, **15**, 278–281.

Jose, P.A., Logan, A.G., Slotkoff, L.M., Lilienfield, L.S., Calcagno, P.L. and Eisner, G.M. (1971) Intrarenal blood flow distribution in canine puppies. *Pediatric Research*, **5**, 335–344.

Jost, A. (1953) Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Progress in Hormone Research*, **8**, 379–413.

Jost, A. (1954) Hormones in the development of the foetus. *Cold Spring Harbor Symposia on Quantitative Biology*, **19**, 167–181.

Jost, A. and Policard, A. (1948) Contribution expérimentale a l'étude du développement prenatal du poumon chez le lapin. *Archives d' Anatomie Microscopique et de Morphologie Expérimentale (Paris)*, **37**, 323–332.

Kadowaki, H., Bibb, P.C. and Knox, W.E. (1983) Capacity of cyclic urea synthesis in neonatal rat liver. *Biology of the Neonate*, **44**, 21–27.

Kagen, L.J. (1973) *Myoglobin*, Columbia University Press, New York.

Kalpaktsogrou, P.K. and Emery, J.L. (1965) Human bone marrow during the last three months of intrauterine life. *Acta Haematologica*, **34**, 228–238.

Kaplan, A. and Friedman, M. (1942) Studies concerning the site of renin formation in the kidney. III. The apparent site of renin formation in the tubules of the mesonephros and metanephros of the hog foetus. *Journal of Experimental Medicine*, **76**, 307–316.

Katz, F.H., Beck, P. and Makowski, E.L. (1974) The renin–aldosterone system in mother and fetus at term. *American Journal of Obstetrics and Gynecology*, **118**, 51–55.

Kaufman, S. (1992) Organogenesis and histologic development of the liver, in *Fetal and Neonatal Physiology*, (eds R. Polin and W. Fox), W.B. Saunders, Philadelphia, pp. 1085–1094.

Kazimierczak, J. (1971) Development of the renal corpuscle and the juxtaglomerular apparatus. *Acta Pathologica, Microbiologica et Immunologica, Scandinavica*, Section A, Suppl. 218.

Kelley, R. (1973) An ultrastructural and cytochemical study of developing small intestine in man. *Journal of Embryology and Experimental Morphology*, **29**, 411–430.

Kervan, A. and Girard, J.R. (1974) Glucose induced increase of plasma insulin in the rat foetus *in utero*. *Journal of Endocrinology*, **62**, 545–551.

Kinmond, S., Aitchinson, T.C., Holland, B.M., Jones, J.G., Turner, T.L. and Wardrop, C.A. J. (1993) Umbilical cord clamping and preterm infants: a randomised trial. *British Medical Journal*, **306**, 172–175.

Kinsella, J.P., Neish, S.R., Shaffer, E. and Abman, S.H. (1992). Low dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet*, **340**, 919–820.

Kiser, T.E., Convey, E.M., Lin, Y.C., Oxender, W.D. (1975) Luteinizing hormone and androgens in the bovine fetus after gonadotropin-

releasing hormone. *Proceedings of the Society of Experimental Biology and Medicine*, **149**, 785–789.

Kitterman, J.A. (1986) Physical factors and fetal lung growth, in *Respiratory Control and Lung Development in the Fetus and Newborn*, (eds B.M. Johnston and P.D. Gluckman), Perinatology Press, Ithaca, New York, pp. 64–85.

Kitterman, J.A., Ballard, P.L., Clements, J.A., Mescher, G.J. and Tooley, W.H. (1979) Tracheal fluid in fetal lambs: spontaneous decrease prior to birth. *Journal of Applied Physiology*, **47**, 985–989.

Kleinman, L.I. and Lubbe, R.J. (1972) Factors affecting the maturation of glomerular filtration rate and renal plasma flow in the newborn dog. *Journal of Physiology*, **223**, 395–409.

Koldovsky, O. (1970) Digestion and absorption during development, in *Physiology of the Perinatal Period*, (ed. U. Stave), Appleton Century Crofts, New York, pp. 379–415.

Koletzko, B., Schmidt, E., Brenner, H., Haug, M. and Garzer, G. (1989) Effects of dietary long-chain polyunsaturated fatty acids on the essential fatty acid status of premature infants. *Journal of Pediatrics*, **148**, 669–675.

Krause, W.J., Cutts, H.J. and Leeson, C.R. (1979) Morphological observations on the mesonephros in the postnatal oppossum, *Didelphis virginiana*. *Journal of Anatomy*, **129**, 377–397.

Kronquist, K.E., Crandall, B.F. and Tabsh, K.M. (1984) Characterization of fetal urinary proteins at midgestion and term. *Biology of the Neonate*, **46**, 267–275.

Kühl, P.G., Cotton, R.B., Schweer, H. and Seyberth, H.W. (1989) Endogenous formation of prostanoids in neonates with PPH. *Archives of Disease in Childhood*, **64**, 949–952.

Lafeber, H.N. (1981) Experimental intrauterine growth retardation in the guinea pig. *PhD Thesis*, Erasmus University.

Lajeunesse, D. and Brunette, N.G. (1988) Sodium gradient–dependent phosphate transport in placental brush border membrane vesicles. *Placenta*, **9**, 117–129.

Leat, W.M.F. (1966) Fatty acid composition of the plasma lipids of newborn and maternal ruminants. *Biochemical Journal*, **98**, 598–603.

Lebenthal, E. and Lee, P.C. (1980) Glucoamylase and disaccharidase activities in normal subjects and in patients with mucosal injury of the small intestine. *Journal of Pediatrics*, **97**, 389–393.

Lebenthal, E., Lee, P.C. and Heitlinger, L.A. (1983) Impact of development of the gastrointestinal tract on infant feeding. *Journal of Pediatrics*, **102**, 1–9.

Leeson, T.S. and Baxter, J.S. (1957) The correlation of structure and function in the mesonephros and metanephros of the rabbit. *Journal of Anatomy*, **91**, 383–390.

Leitch, I., Hyteen, F.C. and Billewicz, W.Z. (1959) The maternal and neonatal weights of some mammalia. *Proceedings of Zoological Society (London)*, **133**, 11–28.

Lemons, J.A., Adcock, E.W., Jones, D., Naughton, H.A., Meshchia, G. and Battaglia, F.C. (1976) Umbilical uptake of amino acids in the unstressed fetal lamb. *Journal of Clinical Investigation*, **59**, 1428.

Lemons, J., Vargas, P. and Delaney, J. (1981) Infant of the diabetic mother: Review of 225 cases. *Obstetrics and Gynecology*, **57**, 187–192.

de Lemos, C. (1977) The ultrastructure of endocrine cells in the corpus of the stomach of human fetuses. *American Journal of Anatomy*, **148**, 359–384.

Lev, R. and Orlic, D. (1973) Uptake of protein in swallowed amitotic fluid by monkey fetal intestine *in utero*. *Gastroenterology*, **65**, 60–68.

Lev, R., Siegel, H.I. and Bartman, J. (1972) Histochemical studies of developing human fetal small intestine. *Histochemie*, **29**, 103–119.

Levene, M.I. (1992) Development of audit measures and guidelines for good practice in the management of neonatal respiratory distress syndrome. *Archives of Disease in Childhood*, **67**, 1221–1227.

Lichtenberger, L. and Johnson, L.R. (1974) Gastrin in the ontogenic development of the small intestine. *American Journal of Physiology*, **227**, 390–395.

Liggins, G. (1990) Obstetric and paediatric collaboration to reduce morbidity after preterm birth. *British Journal of Obstetrics and Gynaecology*, **97**, 1–3.

Lind, T. (1978) The biochemistry of amitotic fluid, in *Amitotic fluid – Research and Clinical Application*, (eds D.V.I. Fairweather and T.K.A.B. Eskes) Amsterdam, Excerpta Medica.

Lind, T. (1981) Amitotic fluid, *Geigy Scientific Tables* (eds K. Diem and C. Lenter) Macclesfield, UK.

Lindblad, B.S. (1976) Protein and amino acid metabolism during fetal development, in *Fetal Physiology and Medicine*, (eds R.W. Berad and P.W. Nathanielsz), W.B. Saunders, London, pp. 80–104.

Linderkamp, O., Ozanne, P., Wu, P.Y.K. and Meiselman, H.J. (1984a) Red blood cell aggre-

gation in preterm and term neonates and adults. *Pediatric Research*, **18**, 1356–1360.

Linderkamp, O., Versmold, H.T., Riegel, K.P. and Betke, K. (1984b) Contributions of red cells and plasma to blood viscosity in preterm and full-term infants and adults. *Pediatrics*, **74**, 45–51.

Linderkamp, O., Gunter, M., Hilt, W. and Vargas, V.M. (1986) Erythrocyte deformability in the fetus, preterm and term neonate. *Pediatric Research*, **20**, 93–96.

Lingwood, B., Hardy, K.J., Coghlan, J.P. and Wintour, E.M. (1978a) Effect of aldosterone on urine composition in the chronically-cannulated ovine fetus. *Journal of Endocrinology*, **76**, 553–554.

Lingwood, B., Hardy, K.J., Horacek, T., McPhee, M.L., Scoggins, B.A. and Wintour, E.M. (1978b) The effects of antidiuretic hormone on urine flow and composition in the chronically-cannulated ovine fetus. *Quarterly Journal of Experimental Physiology*, **63**, 315–330.

Long, W. and Henry, G. (1992) Autonomic and central neural regulation of fetal cardiovascular function, in *Fetal and Neonatal Physiology*, (eds R. Polin and W. Fox), W B Saunders, Philadelphia, pp. 629–645.

Lucarelli, G., Howard, D. and Stohlman, F. (1964) Regulation of erythropoiesis. XV. Neonatal erythropoiesis and the effect of nephrectomy. *Journal of Clinical Investigation*, **43**, 2195–2203.

Lucas, A. (1991) Programming by early nutrition in man, in *The Childhood Environment and Adult Disease*, (eds G.R. Bock and J. Whelan), Wiley, Chichester, pp. 38–55.

Lucas, A., Bloom, S. and Aynsley-Green, A. (1978) Metabolic and endocrine events at the time of first feed of human milk in preterm and term infants. *Archives of Disease in Childhood*, **53**, 731–736.

Lucas, A., Christofides, N., Adrian, T., Bloom, S. and Aynsley-Green, A. (1979) Fetal distress, meconium and motilin. *Lancet*, **i**, 718.

Lucas, A., Bloom, S.R. and Aynsley-Green, A. (1986) gut hormones and 'minimal enteral feeding'. *Acta Paediatrica Scandinavica*, **75**, 719–723.

Lumbers, E.R. and Stevens, A.D. (1983) Changes in fetal renal function in response to infusion of a hyperosmotic solution of mannitol to the ewe. *Journal of Physiology*, **343**, 439–446.

MacDonald, A.A. (1979) Patterns of endocrine change in the pig foetus. *Animal Reproduction Science*, **2**, 289–304.

MacDonald, A.A. (1983) Blood pressure control and the developing pig fetus. *PhD Thesis*, Rijksuniversiteit of Utrecht.

MacDonald, A.A., Colenbrander, B. and Van Vorstenbosch, C.J.A.H.V. (1982) Physiology and endocrinology of the foetus in late gestation, in *Control of Pig Reproduction*, (eds D.J.A. Cole and G.R. Foxcroft), Butterworths, London, pp. 377–404.

MacDonald, D., Grant, A., Sheridan-Pereira, M., Boylan, P. and Chalmers, I. (1985) The Dublin randomized controlled trial of intrapartum fetal heart rate monitoring. *American Journal of Obstetrics and Gynecology*, **152**, 524–539.

MacDonald, M.S. and Emery, J.L. (1959) The late intrauterine and postnatal development of human renal glomeruli. *Journal of Anatomy*, **93**, 331–340.

McCance, R.A. (1972) The role of the developing kidney in the maintenance of internal stability. *Journal of the Royal College of Physicians*, **6**, 235–245.

McCance, R.A. and Stanier, M. (1960) The function of the metanephros of foetal rabbits and pigs. *Journal of Physiology*, **151**, 470–483.

McCance, R.A. and Widdowson, E.M. (1957) New thoughts on renal function in the early days of life. *British Medical Bulletin*, **13**, 3–6.

McCance, R.A. and Widdowson, E.M. (1960) The acid–base relationships of the foetal fluids of the pig. *Journal of Physiology*, **151**, 484–490.

McCance, R.A. and Young, W.F. (1941) The secretion of urine by newborn infants. *Journal of Physiology*, **99**, 265–282.

McClean, P. and Weaver, L. (1993) Ontogeny of human pancreatic exocrine function. *Archives of Disease in Childhood*, **68**, 62–65.

McIntosh, D. and Isaacs, D. (1993) Varicella zoster virus infection in pregnancy. *Archives of Disease in Childhood*, **68**, 1–2.

McNinch, A.W. and Tripp, J.H. (1991) Haemorrhagic disease of the newborn in the British Isles: two year prospective study. *British Medical Journal*, **303**, 1105–1109.

Maiback, H.I. and Boisits, E.K. (1982) *Neonatal Skin: Structure and Function*, Marcel Dekker, New York.

Marino, L.R., Blumer, J.L. and Halpin, T.C. (1983) Prostaglandin E concentration in gastric secretions of critically ill full term and premature infants. *Pediatric Research*, 17, 669–671.

Marlow, N. (1992) Do we need an Apgar score? *Archives of Disease in Childhood*, **67**, 765–767.

Marodi, L., Leijh, P.C.F. and Van Furth, R. (1984)

Characteristics and functional capacities of human cord blood granulocytes and monocytes. *Pediatric Research*, **18**, 1127–1131.

Martin, A.A., Kapoor, R. and Scroop, G.C. (1987) Hormonal factors in control of heart rate in hypoxic fetal and neonatal sheep. *Journal of Developmental Physiology*, **9**, 465–480.

de Martino, C. and Zamboni, L. (1966) A morphologic study of the mesonephros of the human embryo. *Journal of Ultrastructure and Molecular Structure Research*, **16**, 399–427.

Mathews, T. and Warshaw, J. (1979) Relevance of the gestational age distribution of meconium passage *in utero*. *Pediatrics*, **64**, 30–31.

Matoth, Y. and Zaizov, R. (1971) Regulation of erythropoiesis in the fetal rat, in *Erythropoiesis. Regulatory Mechanisms and Developmental Aspects*, (ed. Y. Matoth), Academic Press, New York, pp. 17–21.

Matson, J.R., Stokes, J.B. and Robillard, J.E. (1981) Effects of inhibition of prostaglandin synthesis on fetal renal function. *Kidney International*, **20**, 621–627.

Mears, G.J. and Van Petten, G.R. (1977) Fetal absorption of drugs from the amitotic fluid. *Proceedings of the Western Pharmacological Society*, **20**, 109–114.

Meberg, A., Haga, P. and Johanesen, M. (1980) Plasma erythropoietin levels in mice during the growth period. *British Journal of Haematology*, **45**, 569–574.

Mellor, D.J. (1980) Investigation of fluid spaces of the sheep conceptus, in *Animal Models in Fetal Medicine*, (ed. P.W. Nathanielsz), Elsevier/North Holland Biochemical Press, Amsterdam, pp. 59–106

Mellor, D.J. (1984) Investigation of fetal growth in sheep, in *Animal Models in Fetal Medicine (IV), Monographs in Fetal Physiology 5*, (ed. P.W. Nathanielsz), Elsevier/North Holland Biochemical Press, Amsterdam, pp. 150–175.

Mellor, D.J. and Slater, J.S. (1971) Daily changes in amniotic and allantoic fluid during the last three months of pregnancy in conscious, unstressed ewes with catheters in their foetal fluid sac. *Journal of Physiology*, **217**, 573–604.

Menard, D. and Arsenault, P. (1985) Explant culture of human fetal small intestine. *Gastroenterology*, **88**, 691–700.

Merlet-Benichou, C. and de Rouffignac, C. (1977) Renal clearance studies in fetal and young guinea pigs: effect of salt loading. *American Journal of Physiology*, **232**, F178–F185.

Merlet-Benichou, C., Peggorier, M. and Muffat-Joly, M. (1981) Functional and morphologic patterns of renal maturation in the developing guinea pig. *American Journal of Physiology*, **241**, F618–F624.

Meschia, G., Cotter, J.R., Makowski, E.L. and Barron, D.H. (1967) Simultaneous measurements of uterine and umbilical blood flows and oxygen uptakes. *Quarterly Journal of Experimental Physiology*, **52**, 1–18.

Metcalfe, J., Moll, W., Bartels, H., Hilpert, P. and Parer, J. (1965) Transfer of carbon monoxide and nitrous oxide in the artificially perfused sheep placenta. *Circulation Research*, **16**, 95–101.

Metcalfe, J., Bartles, H. and Moll, W. (1967) Gas exchange in the pregnant uterus. *Physiological Reviews*, **47**, 782–838.

Milner, A.D. (1993) How does exogenous surfactant work? *Archives of Disease in Childhood*, **68**, 253–254.

Milner, A.D. and Vyas, H. (1982) Lung expansion at birth. *Journal of Pediatrics*, **101**, 879–886.

Milner, R.D.G. and Hales, C.N. (1965) Effect of intravenous glucose on maternal and umbilical cord insulin. *British Medical Journal*, **i**, 284–286.

Mistretta, C.M. and Bradley, R.M. (1975) Taste and swallowing *in utero*: a discussion of fetal sensory function. *British Medical Bulletin*, **31**, 80–84.

Moessinger, A.C., Collins, M., Blanc, W., Rey, H. and James, L. (1986) Oligohydramnios induced lung hypoplasia: the influence of timing and duration of gestation. *Pediatric Research*, **20**, 951–954.

Moessinger, A.C., Fox, H., Higgins, A., Rey, H. and Haideri, M.A. (1987) Fetal breathing movements are not a reliable predictor of continued lung development in pregnancies complicated by oligohydramnios. *Lancet*, **2**, 1297–1300.

Moll, I., Moll, R. and Franke, W.W. (1986) Formation of epidermal and dermal merkel cells during human fetal skin development. *Journal of Investigative Dermatology*, **87**, 779–787.

Molteni, A., Rahill, W.J. and Koo, J.H. (1974) Evidence for a vasopresser substance (renin) in human foetal kidneys. *Laboratory Investigation*, **30**, 115–118.

Morales, W., O'Brien, W., Angel, J., Knuppel, R. and Sawai, S. (1989) Fetal lung maturation. The combined use of corticosteroids and thyrotropin releasing hormone. *Obstetrics and Gynecology*, **73**, 111–116.

Mott, J. C. (1985) Humoral control of the fetal

circulation, in *Physiological Development of the Fetus and Newborn*, (eds C.T. Jones and P.W. Nathanielsz), Academic Press, London, pp. 113–121.

Mount, L.E. (1979) *Adaptation to Thermal Environment*, Edward Arnold, London.

Moxey, P.C. and Trier, J.S. (1979) Development of villus absorptive cells in the human fetal small intestine: a morphological and morphometric study. *Anatomical Record*, **195**, 463–482.

Mull, M. and Hathaway, W.E. (1970) Altered platelet function in neonates. *Pediatric Research*, **4**, 229–239.

Mulvihill, S.J., Stone, M.M., Debas, H.T. and Fonkalsrud, E.W. (1985) The role of amniotic fluid in fetal nutrition. *Journal of Pediatric Surgery*, **20**, 668–672.

Muraca, M., Blanckaert, N., Rubaltelli, F.F. and Fevery, J. (1986) Unconjugated and conjugated bilirubin pigments during perinatal development. 1. Studies on rat serum and intestine. *Biology of the Neonate*, **49**, 90–95.

Murphy, S. and Daniels, V.G. (1979) Postnatal amino acid uptake by the rat small intestine. Changes in membrane transport systems for amino acids associated with maturation of jejunal morphology. *Journal of Developmental Physiology*, **1**, 111–126.

Nagel, M.D. and Nagel, J. (1984) Serum erythropoietic activity in the rat around birth. *Biology of the Neonate*, **46**, 44–47.

Nathanielsz, P.W. (ed) (1980) *Animal Models in Fetal Medicine 1.*, Elsevier, North Holland, Amsterdam.

Naughton, J. (1981) Supply of polyenoic fatty acids to the mammalian brain. *International Journal of Biochemistry*, **13**, 21–32.

Nicholls, D.G. (1984) Thermogenic mechanisms in brown fat. *Physiological Reviews*, **64**, 1–64.

Nicolaides, K.H., Rodeck, C.H., Millar, D.S. and Mibashan, R.S. (1985) Fetal haematology in rhesus isoimmunisation. *British Medical Journal*, **290**, 661–663.

Nickel, R., Schummer, A., Seiferle, E. and Sack, W. (1973) The alimentary canal of the ruminants, in *The Viscera of Domestic Animals*, Springer Verlag, New York, pp. 147–175.

Nugteren, D.H., Christ-Hazelhof, E., van der Beek, A. and Houtsmuller, U.M.T. (1985) metabolism of linoleic acid and other essential fatty acids in the epidermis of the rat. *Biochimica et Biophysica Acta*, **834**, 429–436.

O'Callaghan, C. and Stephenson, T. (1992) *Pocket Paediatrics*, Churchill Livingstone, Edinburgh.

Oh, W., Oh, M.A. and Lind, J. (1966) Renal function and blood volume in newborn infants related to placental transfusion. *Acta Paediatrica Scandinavica*, **56**, 19721210.

Oka, Y., Ghishan, F.K. and Greene, H.I. (1983) Effect of mouse epidermal growth factor/urogastrone on the functional maturation of rat intestine. *Endocrinology*, **112**, 94021944.

Olver, R.E. (1983) Fluid balance across the fetal alveolar epithelium. *American Review of Respiratory Disease*, **127**, 533–536.

Olver, R.E. and Strang, L.B. (1974) Ion fluxes across the pulmonary epithelium and the secretion of lung liquid in the foetal lamb. *Journal of Physiology*, **241**, 327–357.

Orchard, M., Lewis, P., Ongari, M., Waddell, K. and Blair, I. (1983) Free 5,8,11-eicosatrienoic acid in human umbilical artery, in *Prostacyclin in Pregnancy*, (eds P. Lewis, S. Moncada and J. O'Grady), Raven Press, New York, pp. 59–63.

OSIRIS Collaborative Group (1992) Early versus delayed administration of a synthetic surfactant – the judgement of OSIRIS. *Lancet*, **ii**, 1363–1369.

Palme-Kilander, C., Tunell, R. and Chiwei, Y. (1993) Pulmonary gas exchange immediately after birth in spontaneously breathing infants. *Archives of Disease in Childhood*, **68**, 6–10.

Parmley, T.H. and Seeds, A.E. (1970) Fetal skin permeability to isotopic water (THO) in early pregnancy. *American Journal of Obstetrics and Gynecology*, **108**, 128–131.

Parry, J.S. and Stanier, M.W. (1962) The rate of flow of urine in fetal pigs. *Journal of Physiology*, **161**, 344–350.

Pasqualini, J.R., Sumida, C. and Gelly, C. (1972) Mineralocorticosteroid receptors in the foetal compartment of the guinea pig. *Journal of Steroid Biochemistry*, **3**, 543–554.

Pasqualini, J.R., Sumida, C., Gelly, C. and Nguyen, B.L. (1976) Specific [^{3}H]-estradiol binding in the fetal uterus and testis of guinea pig. Quantitative evolution of [^{3}H]-estradiol receptors in the different tissues (kidney, lung, uterus and testis) during fetal development. *Journal of Steroid Biochemistry*, **7**, 1031–1038.

Pasqualini, J.R., Sumida, C., Gelly, C. and Nguyen, B.L. (1977) A general view of the quantitative evaluation of cytosol and nuclear steroid hormone receptors in the fetal compartment of

the guinea pig. *Journal of Steroid Biochemistry*, **8**, 445–451.

Pearson, H.A. (1966) Recent advances in hematology. *Journal of Pediatrics*, **69**, 466–482.

Peckham, C. and Logan, S. (1993) Screening for toxoplasmosis during pregnancy. *Archives of Disease in Childhood*, **68**, 3–5.

Peeters, L.L.H., Sheldon, R.E. and Jones, M.D. (1979) Blood flow to fetal organs as a function of arterial oxygen content. *American Journal of Obstetrics and Gynecology*, **135**, 637–646.

Pesonen, E., Haavisto, H., Ammala, P. and Teramo, K. (1983) Intrauterine hydrops caused by premature closure of the foramen ovale. *Archives of Disease in Childhood*, **58**, 1015–1016.

Peters, M.T. and Nicolaides, K.H. (1990) Cordocentesis for the diagnosis and treatment of human parvovirus infection. *Obstetrics and Gynecology*, **75**, 501–504.

Phillips, A., Dubin, J. and Raye, J. (1981) Metabolic response of the fetal lamb to endogenously released insulin. *American Journal of Obstetrics and Gynecology*, **139**, 441–445.

Pitkin, R.M. and Reynolds, W.A. (1975) Fetal ingestion and metabolism of amitotic fluid protein. *American Journal of Obstetrics and Gynecology*, **123**, 356–361.

Pointis, G. and Latreille, M.T. (1986) Absence of LHRH effect on testosterone production by Leydig cells from fetal mouse testis. *Journal of Steroid Biochemistry*, **24**, 307–310.

Ponte, J. and Purves, M. (1973) Types of different nerve activity which may be measured in the vagus nerve of the sheep fetus. *Journal of Physiology*, **229**, 51–76.

Ponzilius, K.H., Parvizi, N., Elaesser, F. and Ellendorf, F. (1986) Ontogeny of secretory patterns of LH release and effects of gonadectomy in the chronically-catheterized pig fetus and neonate. *Biology of Reproduction*, **34**, 602–612.

Posner, B.I. (1974) Insulin receptors in human and animal placental tissue. *Diabetes*, **23**, 209–217.

Potau, N., Riudor, E. and Balla (1981) Insulin receptors in human placenta in relation to foetal weight, growth and gestational age. *Pediatric Research*, **15**, 798–802.

Potter, E.L. and Thierstein, S.T. (1943) Glomerular development in the kidneys as an index of fetal maturity. *Journal of Pediatrics*, **22**, 695–706.

Precht, H., Christopherson, J., Hensel, H. and Larcher, W. (1973) *Temperature and Life*, Springer-Verlag, Berlin.

Prechtl, H.F.R. (1988) Assessment of fetal neurological function and development, in *Fetal and Neonatal Neurology and Neurosurgery*, (eds M.I. Levene, M.J. Bennett and J. Punt), Churchill Livingstone, Edinburgh.

Prentice, A. (1991) Can maternal dietary supplements help in preventing infant malnutrition. *Acta Paediatrica Scandinavica*, Suppl. 374, 67–77.

Pritchard, J.A. (1965) Deglutition by normal and anencephalic fetuses. *Obstetrics and Gynecology*, **25**, 289–297.

Purves, M. (1981) The neural control of respiration before and after birth. *Perinatal Medicine*, **4**, 299–336.

Raiha, N.C.R. (1971) The development of some enzymes of amino acid metabolism in the human liver, in *Metabolic Processes in the Foetus and Newborn Infant*, (eds J.H.P. Jonxis, H.K.A. Visser and J.A. Troelstra) Liecka Stenfert, Kroese, Leden, N.V., pp. 26–34.

Raiha, N.C.R. and Suinhkonen, J. (1968a) Development of urea-synthesizing enzymes in human liver. *Acta Paediatrica Scandinavica*, **57**, 121–124.

Raiha, N.C.R. and Suinhkonen, J. (1968b) Factors influencing the development of urea-synthesizing enzymes in rat liver. *Biochemical Journal*, **107**, 793–797.

Randall, G.C.B. and L'Ecuyer, C. (1976) Tissue glycogen and blood glucose and fructose levels in the pig fetus during the second half of gestation. *Biology of the Neonate*, **28**, 74–82.

Raux-Eurin, M.C., Pharm Huu Trung, M.T. and Marrec, D. (1977) Plasma aldosterone concentration during the newborn period. *Pediatric Research*, **11**, 182–185.

Read, L.C., Upton, F.M., Francis, G.L., Wallace, J.C., Dahlenberg, G.W. and Ballard, F.J. (1984) Changes in the growth-promoting activity of human milk during lactation. *Pediatric Research*, **19**, 213.

Rennie, J. and Cooke, R. (1992) Prolonged low dose indomethacin for the persistent ductus arteriosus of prematurity. *Archives of Disease in Childhood*, **66**, 55–58.

Resko, J.A. and Ellingwood, W.E. (1985) Negative feedback regulation of gonadotrophin secretion by androgens in fetal rhesus macaques. *Biology of Reproduction*, **33**, 346–352.

Rhoda, J., Valens, M., Edwards, D.A. and Roffi, J. (1987) Effect of progesterone on the testosterone and estradiol levels in the hypothalamus of rats. *Biology of the Neonate*, **51**, 255–259.

Ricquier, D. (1984) Factors affecting brown adi-

pose tissue activity in animals and man. *Clinics in Endocrinology and Metabolism*, **13**, 501–520.

Rigaudière N. and Després, G. (1986) Maternal testosterone and sexual differentiation of the male guinea pig foetus. *Reproduction, Nutrition, Development*, **26**, 791–800.

Rivers, P. and Frankel, T. (1981) Essential fatty acid deficiency. *British Medical Bulletin*, **37**, 59–64.

Robertson, A.F. and Sprecher, H. (1967) Human placental lipid metabolism. III. Synthesis and hydrolysis of phospholipids. *Lipids*, **2**, 403–405.

Robertson, A. F. and Sprecher, H. (1968) A review of human placental lipid metabolism. *Acta Paediatrica Scandinavica*, Suppl. 183, 3–17.

Roberton, N.R.C. (1986) *Textbook of Neonatology*, Churchill Livingstone, Edinburgh.

Robillard, J.E. and Weitzman, R.E. (1980) Developmental aspects of the fetal renal response to exogenous arginine vasopressin. *American Journal of Physiology*, **238**, F407–F414.

Robillard, J.E., Matson, J.R., Sessions, C. and Smith, F.J. (1979) Developmental aspects of renal tubular reabsorption of water in the lamb fetus. *Pediatric Research*, **13**, 606–610.

Robillard, J. and Nakamura, K. (1988) Neurohormonal regulation of renal function during development. *American Journal of Physiology*, **254**, F771–F779.

Robillard, J.E., Session, C., Kennedey, R.I., Hamel-Robillard, L. and Smith, F.G. (1977) Interrelationships between GFR and renal transport of sodium and chloride during foetal life. *American Journal of Obstetrics and Gynecology*, **128**, 727–734.

Robillard, J.E., Ramberg, E., Sessions, C., Consamus, B., van Orden, D., Weismann, D. and Smith, F.J. (1980) Role of aldosterone on renal sodium and potassium excretion during fetal life and newborn period. *Developmental Pharmacology and Therapeutics*, **1**, 201–216.

Robillard, J., Nakamura, K., Matherne, G. and Jose, P. (1988) Renal haemodynamics and functional adjustment to postnatal life. *Seminars in Perinatology*, **12**, 143–150.

Robinson, A. and Williamson, D. (1980) Physiological role of ketone bodies as substrates and signals in mammalian tissues. *Physiological Reviews*, **60**, 143–189.

Rodewald, R. (1973) Intestinal transport of antibodies in newborn rat. *Journal of Cell Biology*, **58**, 189–211.

Rosenthal, P. (1992) Bilirubin metabolism in the fetus and neonate, in *Fetal and Neonatal Physiology*, (eds R. Polin and W. Fox), W.B. Saunders, Philadelphia, pp. 1154–1159.

Ross, M. G. (1988) Fetal ANF and arginic vasopressive responses to hyperosmolality and hypervolaemia. *Pediatric Research*, **24**, 318–321.

Ross, M.G., Ervine, M.G., Lam, R.W., Castro, L., Leake, R.D. and Fisher, D.A. (1987). Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus. *American Journal of Obstetrics and Gynecology*, **157**, 1292–1297.

Roux, J.F. and Myers, R.E. (1974) *In vitro* metabolism of palmitic acid and glucose in the developing tissue of the rhesus monkey. *American Journal of Obstetrics and Gynecology*, **118**, 385–392.

Rudolph, A. (1983) Hepatic and ductus venous blood flows during fetal life. *Hepatology*, **3**, 254–258.

Rudolph, A.M. and Heyman, M.A. (1980) Methods for studying the circulation of the fetus *in utero*, in *Animal Models in Fetal Medicine*, (ed. P.W. Nathanielsz), Elseveir, North Holland, Amsterdam, pp. 1–57.

Rudolph, A. and Rudolph, C. (1992) Fetal and postnatal hepatic vasculature and blood flow, in *Fetal and Neonatal Physiology*, (eds R. Polin and W. Fox), W.B. Saunders, Philadelphia, pp. 1095–1102.

Ruoslahti, E. and Hirai, H. (1976) Alphafetoprotein. *Scandinavian Journal of Immunology*, Suppl. 8, 3–26,

Rurak, D., Richardson, B., Patrick, J., Carmichael, L. and Homan, J. (1990) Oxygen consumption in the fetal lamb during sustained hypoxemia with progressive acidemia. *American Journal of Physiology*, **258**, R1108–R1115.

Rutter, N. and Hull, D. (1979a) Response of babies to a warm environment. *Archives of Disease in Childhood*, **54**, 178–183.

Rutter, N. and Hull, D. (1979b) Water loss from the skin of term and preterm babies. *Archives of Disease in Childhood*, **54**, 858–868.

Sacher, G.E. and Staffeldt, E.F. (1974) Relation of gestation time to birth weight for placental mammals: Implications for the theory of vertebrate growth. *American Naturist*, **198**, 593–615.

Sadava, D., Depper, M., Gilbert, M., Bernard, B. and McCabe, E.R.B. (1987) Development of enzymes of glycerol metabolism in human fetal liver. *Biology of the Neonate*, **52**, 26–32.

Said, H.M., Ghishan, F.K., Greene, H.L. and Hollander, D. (1985) Developmental maturation

of riboflavin intestinal transport in the rat. *Pediatric Research*, **19**, 1175–1178.

Saigal, S., O'Neil, A., Surainder, Y., Chau, L.B. and Usher, R. (1972) Placental transfusion in hyperbilirubinemia in the premature. *Paediatrics*, **49**, 406–419.

Saraux, B. and Girad-Globa, A. (1982) Development of pancreatic enzymes in fetal and suckling rats with emphasis on lipase and colipase. *Journal of Developmental Physiology*, **4**, 121–137.

Schlondorff, D., Weber, H., Trizna, H. and Fine, L.G. (1978) Vasopressin responsiveness of renal adenylate cyclase in newborn rats and rabbits. *American Journal of Physiology*, **234**, F16–F21.

Schmidt, U. and Horster, M. (1977) Na-K-activated ATPase: activity maturation in rabbit nephron segments dissected *in vitro*. *American Journal of Physiology*, **233**, F55–F60.

Sengel, P. (1976) *Morphogenesis of Skin*, Cambridge University Press, Cambridge, UK.

Senterre, J. and Salle, B. (1988) Renal aspects of calcium and phosphorus metabolism in preterm infants. *Biology of the Neonate*, **53** (4), 220–229.

Shaffer, S.G. (1987) Extracellular fluid volume changes in very low birthweight infants during first 2 postnatal months. *Journal of Pediatrics*, **111**, 124.

Shelley, H.J. (1961) Glycogen reserves and their changes at birth. *British Medical Bulletin*, **17**, 137–144.

Shelley, H.J. (1979) Transfer of carbohydrate. in *Placental Transfer*, (eds G.V.P. Chamberlain and A.W. Wilkinson), Pitman Medical, Kent, UK, pp. 118–142.

Shulkes, A. Chick, P. and Hardy, K.J. (1982) Fetal and maternal production and metabolism of gastrin in sheep. *Journal of Endocrinology*, **94**, 183–189.

Shulkes, A. Chick, P., Hardy, K.J., Robinson, P. and Trahair, J. (1985) Ontogeny of gastric acidity in the ovine fetus. *Journal of Developmental Physiology*, **7**, 195–206.

Sibley, C.P. and Boyd, R.D.H. (1988) Control of transfer across the mature placenta. *Oxford Reviews of Reproductive Biology*, **10**, 382–435.

Sibley, C.P., Ward, B.S. and Grazier, J.D. (1986) Electrical activity and sodium transport across *in vitro* pig placenta. *American Journal of Physiology*, **250**, R474–R484.

Siegel, S.R. (1982) Hormonal and renal interaction in body fluid regulation in the newborn infant, in *Clinics in Perinatology*, Vol. 9, (ed. P.W.W. Wu), W.B. Saunders, Philadelphia, pp. 535–557.

Siegel, S.R. and Fisher, D.A. (1980) Ontogeny of the renin–angiotensin–aldosterone system in the fetal and newborn lamb. *Pediatric Research*, **14**, 99–102.

Siiteri, P.K. and Wilson, J.D. (1974) Testosterone formation and metabolism during male sexual differentiation in the human embryo. *Journal of Clinical Endocrinology and Metabolism*, **38**, 113–125.

Simmons, M., Battaglia, F. and Meschia, G. (1978) *In vivo* effect of insulin on fetal glucose utilization and transplacental glucose transport. *Pediatric Research*, **12**, 90–92.

Simpson, J. and Stephenson, T. (1993) Regulation of extracellular fluid volume in neonates. *Early Human Development*, (In press).

Smith, C.H. (1972) *Blood Diseases of Infancy and Childhood*, C.V. Mosby, St. Louis.

Smith, R.A. and Glomski, C.A. (1977) Embryonic and fetal hemopoiesis in the Mongolian gerbil (*Meriones unguiculatus*). *Anatomical Record*, **189**, 499–518.

Snell, K. and Walker, D.G. (1973) Gluconeogenesis in the newborn rat: the substrates and their quantitative significance. *Enzyme*, **15**, 40–81.

Soifer, S.J. and Heymann, M.A. (1984) Future research directions in persistent pulmonary hypertension of the newborn. *Clinics in Perinatology*, **11**, 745–755.

Soifer, S.J., Loitz, R.D., Roman, C. and Heymann, M.A. (1985a) Leukotriene end organ antagonists increase pulmonary blood flow in fetal lambs. *American Journal of Physiology*, **249**, H570–H576.

Soifer, S.J., Schreiber, M.D., Loitz, R.D., Roman, C. and Heymann, M.A. (1985b) The effects of leukotriene inhibition on the perinatal pulmonary circulation in the lamb, in *The Physiological Development of the Fetus and Newborn*, (eds C.T. Jones and P.W. Nathanielsz), Academic Press, London, pp. 451–456.

Solinger, A.M. (1985) Immature T lymphocytes in human neonatal blood. *Cellular Immunology*, **92**, 115–122.

Spitzer, A. (1982) The role of the kidney in sodium homeostasis during maturation. *Kidney International*, **21**, 539–546.

Spitzer, A. and Brandis, M. (1974) Functional and morphologic maturation of the superficial nephrons. *Journal of Clinical Investigation*, **53**, 279–287.

Spitzer, A. and Edelman, C.M. (1971)

Maturational changes in pressure gradients for glomerular filtration. *American Journal of Physiology*, **221**, 1431–1435.

Stagno, S. (1990) Cytomegalovirus, in *Infectious Diseases of the Fetus and Newborn Infant*, (eds J.S. Remington and J.O. Klein), W.B. Saunders, Philadelphia.

Stamatoyannopoulos, G. and Neinhuis, A.E. (1981) *Hemoglobins in Development and Differentiation*, Alan R. Liss, New York.

Stammers, J.P., Leadon, D.P. and Hull, D. (1987) Fatty acid composition of the plasma lipids of the maternal and newborn horse. *Journal of Reproduction and Fertility*, Suppl.35, 615–622.

Stanier, M. (1960) The function of the mammalian mesonephros. *Journal of Physiology*, **151**, 472–478.

Stanier, M.W. (1972) Development of intrasolute gradients in foetal and postnatal life. *Pflugers Archives*, **2336**, 263–270.

Steer, P., Lucas, A. and Sinclair, J. (1992) Feeding the low birth weight infant, in *Effective Care of the Newborn Infant*, (eds J. Sinclair and M. Bracken), Oxford University Press, Oxford, pp. 94–138.

Stein, S., Cherian, M.G. and Mazur, A. (1971) Biochemistry of rat haemoglobins. *Federation Proceedings*, **30**, 1257.

Stephenson, T.J. (1993) Fetus, in *Essential paediatrics*, (eds D. Hull and D.I. Johnston), 3rd edn, Churchill Livingstone, Edinburgh, pp. 32–43.

Stephenson, T.J. and Broughton-Pipkin, F. (1990) Atrial natriuretic factor: the heart as an endocrine organ. *Archives of Disease in Childhood*, **65**, 1293–1294.

Stephenson, T. and Rutter, N. (1992a) Fluid requirements, in *Intravenous Feeding of the Neonate*, (eds V. Yu and A. Macmahon), Edward Arnold, London, pp. 3–12.

Stephenson, T. and Rutter, N. (1992b) Thermoregulation and fluid balance in the newborn, in *Forfar and Arneil's Textbook of Paediatrics*, (eds N. McIntosh and A. Campbell), Churchill Livingstone, Edinburgh, pp. 158–165.

Stephenson, T.J., Stammers, J.P. and Hull, D. (1990a) Effects of altering umbilical flow and umbilical free fatty acid concentration on transfer of free fatty acids across the rabbit placenta. *Journal of Developmental Physiology*, **15**, 221–227.

Stephenson, T.J., Stammers, J.P. and Hull, D. (1990b) Maternal to fetal transfer of free fatty acids in the 'in situ' perfused rabbit placenta. *Journal of Developmental Physiology*, **13**, 117–123.

Stephenson, T.J., Broughton-Pipkin, F. and Elias-Jones, A. (1991) Factors influencing plasma renin and renin substrate in premature infants. *Archives of Disease in Childhood*, **66**, 1150–1154.

Stephenson, T.J., Stammers, J.P. and Hull, D. (1993a) Placental transfer of free fatty acids: importance of fetal albumin concentration and acid base status. *Biology of the Neonate*, **63**, 273–80.

Stephenson, T.J., Stammers, J.P. and Hull, D. (1993b) Effect of exogenous lipid on placental transfer in the anaesthetized rabbit. *Journal of Physiology*, **53P**, (in press).

Stevens, D.H. (1975) *Comparative Placentation – essays in structure and function*, Academic Press, London.

Stone, R.T. (1981) *In vitro* liver synthesis and serum levels of alpha fetoprotein and albumin in the fetal pig. *Biology of Reproduction*, **24**, 573–580.

Strang, L.B. (1977) *Neonatal Respiration, Physiological and Clinical Studies*, Blackwell Scientific Publications, Oxford.

Stulc, J. (1988) Is there control of solute transport at placental level? *Placenta*, **9**, 19–26.

Sujov, P., Kellerman, L., Zelter, M. and Hochberg, Z. (1984) Plasma and urine osmolality in full-term and pre-term infants. *Acta Paediatrica Scandinavica*, **73**, 722–726.

Sulyok, E., Nemeth, M., Tenyi, I., Csaba, I.F., Varga, F., Gyory, E. and Thurzo, V. (1979) Relationship between maturity, electrolyte balance and the function of the renin–angiotensin–aldosterone system in newborn infants. *Biology of the Neonate*, **35**, 60–65.

Sumida, C. and Pasqualini, J.R. (1979) Determination of cytosol and nuclear estradiol-binding sites in fetal guinea pig uterus by [^{3}H] estradiol exchange. *Endocrinology*, **105**, 406–413.

Tanner, J.M. (1978) *Foetus into Man: Physical Growth from Conception to Maturity*, Open Books, London.

Tarnow-Mordi, W., Shaw, J., Liu, D., Gardner, D. and Flynn, F. (1981) Iatrogenic hyponatraemia of the newborn due to maternal fluid overload: a prospective study. *British Medical Journal*, **283**, 639–642.

Tchernia, G., Mielot, F., Coulombel, L. and Mohandas, N. (1981) Characterization of circulating erythroid progenitor cells in human newborn blood. *Journal of Laboratory and Clinical Medicine*, **97**, 322–331.

Teitel, D. (1992) Physiologic development of the cardiovascular system in the fetus, in *Fetal and Neonatal Physiology*, (eds R. Polin and W. Fox), W.B. Saunders, Philadelphia, pp. 609–619.

Thorburn, G.D., Waters, M.J., Young, I.R., Dolling, M., Buntine, D. and Hopkins, K.S. (1981) Epidermal growth factor: a critical factor in fetal maturation?, in *The Fetus and Independent Life*, Ciba Foundation Symposium, **86**, Pitman, London

Tiedemann, K. (1976) The mesonephros of cat and sheep. Comparative morphological and histochemical studies. *Advances in Anatomy, Embryology and Cell Biology*, **52**, 7–119.

Tomoda, S. Brace, R.A. and Longo, L.D. (1985) Amniotic fluid volume and fetal swallowing rate in sheep. *American Journal of Physiology*, **249**, R133–R138.

Towstoless, M.K., McDougall, J.G. and Wintour, E.M. (1989) Gestational changes in renal responsiveness to cortisol in the ovine fetus. *Pediatric Research*, **26**, 6–10.

Trahair, J.F. and Robinson, P.M. (1986) The development of the ovine small intestine. *Anatomical Record*, **214**, 294–303.

Tsai-Morris, C.H., Knox, G., Luna, S. and Dufau, M.L. (1986) Acquisition of estradiol-mediated regulatory mechanism of steroidogenesis in cultured fetal rat Leydig cells. *Journal of Biological Chemistry*, **261**, 3471–3474.

Ts'ao, C., Green, D. and Schultz, K. (1976) Function and ultrastructure of platelets of neonates: enhanced ristocetin aggregation of neonatal platelets. *British Journal of Haematology*, **32**, 225–233.

Tsuda, F., Nickerson, P.A. and Molteni, A. (1971) Ultrastructural study of developing juxtaglomerular cells in the rat. *Laboratory Investigation*, **25**, 644–652.

Tudehope, D. and Smyth, M. (1979) Is transient tachypnoea of the newborn always a benign condition? *Australian Paediatric Journal*, **15**, 160–165.

Tudvad, F. and Vesterdal, J. (1953) The maximal tubular transport of glucose and para-aminohippurate in premature infants. *Acta Paediatrica Scandinavica*, **42**, 337–345.

Tuvemo, T. (1980) Role of prostaglandins, prostacyclin and thromboxanes in the control of the umbilical–placental circulation. *Seminars in Perinatology*, **4**, 91–95.

Uauy, R., Birch, D., Birch, E., Tyson, J. and Hoffman, D. (1990) Effect of dietary omega–3 fatty acids on retinal function of very-low-birthweight neonates. *Pediatric Research*, **28**, 485–492.

Vanapruks, V. and Prapaitrakul, K. (1989) Water intoxication and hyponatraemic convulsions in neonates. *Archives of Disease in Childhood*, **64**, 734–735.

Van Assche, F.A. and Hoet, J.J. (1984) Endocrine pancreas of the pregnant mother, fetus and newborn, in *Fetal Physiology and Medicine*, (eds R.W. Beard and P.W. Nathanielsz), 2nd edn, Marcel Dekker, New York, pp. 127–152.

Van Assche, F.A. and Robertson, W.B. (eds) (1982) *Fetal Growth Retardation*. Churchill Livingstone, Edinburgh.

Van Dijk, J.P. (1988) Regulatory aspects of placenta iron transfer – a comparative study. *Placenta*, **9**, 215–226.

Varille, V., Nakamura, K., McWeeny, O. and Matherne, G. (1989) Renal hemodynamic response to atrial natriuretic factor in fetal and newborn sheep. *Pediatric Research*, **25**, 291–294.

Vernier, R.L. and Birch-Andersen, A. (1962) Studies of the human fetal kidney. 1. Development of the glomerulus. *Journal of Pediatrics*, **60**, 754–768.

Vernon, R. and Walker, D.G. (1970) Glycerol metabolism in the neonatal rat. *Biochemical Journal*, **118**, 531–536.

Veyssiere, G., Berger, M., Jean-Faucher, C., de Turckheim, M. and Jean, C. (1976) Levels of testosterone in the plasma, gonads and adrenal during fetal development of the rabbit. *Endocrinology*, **99**, 1263–1268.

Veyssiere, G., Berger, M., Jean-Faucher, C., de Turckheim, M. and Jean, C. (1977) Effect of luteinising hormone (LH) and LH-releasing hormone (LHRH) on testosterone production *in vivo* in fetal rabbit testis in late gestation. *Biology of the Neonate*, **32**, 327–330.

Vinter, J. and Hull, D. (1982) Onset of thermogenesis in response to cold in newborn mice. *Biology of the Neonate*, **42**, 145–151.

Vyas, H. (1986) The effect of mode of delivery on lung expansion at birth. *Doctor of Medicine Thesis*, University of Nottingham.

Walker, A. (1984) Physiological control of the fetal cardiovascular system, in *Fetal Physiology and Medicine*, (eds R. Beard and P. Nathanielsz), Marcel Dekker, New York, pp. 287–316.

Warshaw, J.B. (1972) Cellular energy metabolism during fetal development. IV. Fatty acid activation, acetyl transfer and fatty acid oxidation during development of the chick and rat. *Developmental Biology*, **28**, 537–544.

Warshaw, J.B. (1979) Fatty-acid metabolism dur-

ing development. *Seminars in Perinatology*, **3**, 131–139.

Weatherall, D. (1982) *The New Genetics and Clinical Practice*, The Nuffield Provincial Hospitals Trust, London.

Weaver, L. (1992) Breast and gut: the relationship between lactating mammary function and neonatal gastrointestinal function. *Proceedings of the Nutrition Society*, **51**, 155–163.

Weaver, L. and Lucas, A. (1993) Development of bowel habit in preterm infants. *Archives of Disease in Childhood*, **68**, 317–320.

Weston, W.L. (ed.) (1985) *Practical Pediatric Dermatology*. 2nd edn, Little and Brown, Boston.

Widdowson, E. (1950) Chemical composition of newly born animals. *Nature*, **166**, 626–628.

Widdowson, E.M. (1970) The harmony of growth. *Lancet*, **i**, 901–905.

Wigglesworth, J.S. and Desai, R. (1979) Effects on lung growth of cervical cord section in the rabbit fetus. *Journal of Endocrinology*, **3**, 51–65.

Wilkins, B. (1992) Renal function in sick very low birthweight infants. 1. Glomerular filtration rate. *Archives of Disease in Childhood*, **67**, 1140–1145.

Witsett, J.A., Johnson, C.L. and Hawkins, K. (1979) Differences in localisation of insulin receptors and adenylate cyclase in the human placenta. *American Journal of Obstetrics and Gynecology*, **133**, 204–207.

Wright, G.H. (1963) Net transfers of water, sodium, chloride and hydrogen ions across the gastric mucosa of the rabbit foetus. *Journal of Physiology*, **163**, 281–292.

Yoffey, J.M. and Thomas, D.B. (1964) The development of bone marrow in the human fetus. *Journal of Anatomy*, **98**, 463–464.

Young, M. (1979) Transfer of amino acids. (eds G.V.P. Chamberlain and A.W. Wilkinson) Pitman Medical, UK.

Young, S., LeSoëuf, P., Geelhoed, G., Stick, S.M., Turner, K.J. and Landau, L. (1991) The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. *New England Journal of Medicine*, **324**, 1168–1173.

Zamboni, L. and Upadhyay, S. (1981) Ephemeral, rudimentary glomerular structures in the mesonephros of the mouse. *Anatomical Record*, **201**, 641–644.

Zanjani, E.D., Peterson, E.N., Gordon, A.S. and Wasserman, L.R. (1974) Erthropoetin production in the fetus: role of the kidney and maternal anemia. *Journal of Laboratory and Clinical Medicine*, **83**, 281–287.

Zanjani, E.D., Poster, J., Mann, L.J. and Wasserman, L.R. (1977) *Kidney Hormones. Vol. 2 Erythropoietin*. (ed. J.W. Fisher), Academic Press, London, pp.463–493.

Zanjani, E.D., Ascensao, J.L., McGlave, P., Banisadre, M. and Ash, R.C. (1981) Studies on the liver to kidney switch of erythropoietin production. *Journal of Clinical Investigation*, **67**, 1183–1188.

Zimmermann, T., Hummel, L. and Wagner, H. (1986) Quantitative studies on the fetal lipid metabolism in rats: Liver fatty acid esterification and conversion into carbon dioxide, and hepatic output of triglycerides and phospholipids into serum. *Biology of the Neonate*, **49**, 43–50.

Zimmermann, T., Winkler, L., Möller, W., Schubert, M. and Goetze, E. (1979) Synthesis of arachidonic acid in human placenta *in vitro*. *Biology of the Neonate*, **35**, 209–212.

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9.1 INTRODUCTION

The discovery in 1910 (see Liggins, 1988, for historical review) of the dependence of continuing pregnancy in rodents on the corpus luteum and the subsequent isolation of progesterone seemed to provide a sound basis for the mechanism initiating parturition. The 'progesterone withdrawal' hypothesis gained widespread acceptance regardless of whether the maintenance of pregnancy was corpus luteum dependent or placenta dependent. Because the luteotrophic complex of hormones in rodents is of maternal origin, little reason existed to depart from the traditional view that the mechanism resided in the mother rather than the conceptus. However, the 1950s saw increasing awareness of the evidence that fetal genotype in farm animals and certain fetal malformations in both man and farm animals influence the duration of pregnancy. With the unequivocal demonstration in 1967 that hypophysectomy in fetal sheep abolishes the initiation of parturition, the pendulum swung decisively away from maternal control towards fetal control and the fetal endocrine system became the focus of attention of most investigators.

Their convenient size, docility and tolerance of complex surgical procedures in the fetus ensured the sheep was the favoured experimental animal, but hopes that the mechanism elucidated in sheep could be extrapolated across all species were soon dispelled. Broad fundamental differences in the comparative physiology of parturition emerged. At one end of the spectrum lies the sheep, in which the fetal endocrine system dominates all other influences; at the other end lie the primates, in which the fetus itself appears to have only a very minor role. The latter should not be misunderstood as indicating a major maternal role but rather that tissues of the conceptus other than fetus (the placenta and membranes) are important.

Two discoveries in particular have been helpful in determining where in the spectrum a particular species is likely to be. First, prostaglandins, particularly prostaglandin $F_{2\alpha}$, were found not only to activate contractility of the pregnant uterus but also to induce luteolysis. Second, activity of the placental 17α-hydroxylase enzyme, which catalyses the conversion of progesterone or pregnenolone to oestrogen, was shown to be inducible by fetal cortisol, thus providing a link between the fetal hypothalamic–pituitary–adrenal system and the relative production rates of oestrogen and progesterone in those species in which the placental enzyme is present.

Species lying at the 'non-fetal' end of the spectrum have been the most resistant to successful investigation, mainly because the system is paracrine rather than endocrine; the study of the chemical messengers by which the fetal trophoblast of the placenta and membranes communicates directly with the contiguous uterine epithelium (decidua) presents obvious difficulties.

Given a previously unstudied species, designing experiments that will place the individual appropriately in the general classification has become relatively straightforward, requiring an answer to only two questions:

1. Does luteectomy or $PGF_{2\alpha}$ -induced luteolysis lead to parturition?
2. Does the placenta contain an active 17α-hydroxylase?

If the answer to either question is affirmative, further investigations can follow the patterns established for species such as the rabbit and the sheep respectively. If the answers to both questions are negative, further investigations are ill-defined because of the lack of strong hypotheses.

Abbreviations used in chapter:

17α-OH-P_4	17α-hydroxyprogesterone
17α-P_5	17α-hydroxypregnenolone
20α-P_4	20α-hydroxypreg-4-en-3-one (20α-dihydroprogesterone)

20α-ol	5β-pregnane-3α,20α-diol
ACTH	adrenocorticotrophic hormone
BNC	binucleate cells
CG	chorionic gonadotrophin
CL	corpus luteum/corpora lutea
CRF	corticotrophin-releasing factor
DAG	diacylglycerol
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
E/P_4	oestrogen–progesterone ratio
EIPS	endogenous inhibitor of prostaglandin synthase
EMG	electromyogram
EGF	epidermal growth factor
GH	growth hormone
GnRH	gonadotrophin-releasing hormone
hCG	human chorionic gonadotrophin
HDL	high-density lipoprotein
HETE	hydroxyeicosatetraenoic acid
HSD	hydroxysteroid dehydrogenase
IL	interleukin
IP	inositol phosphate
LDL	low-density lipoprotein
LH	luteinizing hormone
LT	leukotriene
MCR	metabolic clearance rate
MPA	medroxyprogesterone acetate
oPL	ovine placental lactogen
P_4	progesterone
P_5	pregnenolone
PAF	platelet-activating factor
PAPSI	pregnancy-associated prostaglandin synthase inhibitor
PG	prostaglandin
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PGEM	prostaglandin E_2 metabolite
PGFM	prostaglandin F metabolite
PGHS	prostaglandin H synthase
PL	placental lactogen
PL	phospholipase
RIA	radioimmunoassay
RU486	mifepristone
TPA	12-*O*-tetradecanoylphorbol 13-acetate
TX	thromboxane

9.2 MAN

9.2.1 INTRODUCTION

The mechanism of initiation of parturition in man is unknown, but several hypotheses have a general acceptance. Foremost among these is the view that the release of eicosanoids underlies the major physiological changes in both the smooth muscle and connective tissue of the pregnant uterus that lead to the onset of parturition. Since neither progesterone (P_4) nor oestrogen concentrations change acutely at term as they do in many other species, other triggers to eicosanoid release are being sought; most favoured for this role is oxytocin which, although unaltered in circulating concentration, may have heightened activity at term by virtue of increased numbers of receptor sites. The eicosanoid mainly responsible for parturition (PGE_2 or $PGF_{2\alpha}$) and the major site of synthesis (amnion or decidua) are subjects of controversy.

Circumstantial evidence from experiments in nature provided by congenital defects of the pituitary or adrenals points to the human fetus having a modulating rather than a controlling role in initiating parturition. Accordingly, attention tends to be directed to the likelihood that the mechanism resides in a paracrine relationship of the fetal membranes and/or placenta and the maternal decidua. Several possible paracrine signals have been proposed but all remain speculative.

This review of human parturition assumes a central role for eicosanoids and considers the evidence supporting this assumption, the known actions of eicosanoids on the uterus, and the sites and control of eicosanoid synthesis. In addition, the possible contributions of hormones other than eicosanoids that act on the uterus independently of interactions

with eicosanoid synthesis are reviewed. It is recognized, however, that the field of smooth muscle physiology is advancing rapidly and that endothelium-derived relaxing factor (EDRF) and endothelin, which are probably important in the paracrine control of vascular smooth muscle, are unexplored as potential agonists for uterine smooth muscle. A major shift in emphasis in human parturitional physiology in the future remains a possibility.

9.2.2 ROLE OF THE FETUS

The weight of circumstantial evidence derived from various congenital malformations and disorders involving fetal endocrine organs is against an active role of the fetus in initiating parturition. A malformation that is associated consistently with early or late delivery in the absence of a mechanical factor such as polyhydramnios has yet to be identified. Lack of the pituitary in anencephaly (W.M. Hcnnebier and Swaab, 1973) or of the hypothalamus (Janigan *et al.*, 1962) is compatible with delivery at term, as is congenital absence of the fetal adrenals (Pakravan *et al.*, 1974). The administration of large doses of corticosteroids to the mother is not associated with preterm delivery, although glucocorticoid activity in fetal blood rises to concentrations at least equal to those of the term fetus (Ballard and Liggins, 1982).

Corticotrophin-releasing factor (CRF) is secreted by the cytotrophoblast and is present in fetal blood (Goland *et al.*, 1986). CRF synthesis is stimulated by glucocorticoids, and a positive-feedback loop involving fetal pituitary ACTH and adrenal cortisol secretion may lead to rising cortisol concentrations prepartum (B.G. Robinson *et al.*, 1988). Quartero and Fry (1989) proposed that CRF potentiated the inotropic effect of oxytocin on strips of human myometrium. The concentration of CRF in maternal plasma increases 50-fold during the third trimester (Sasaki *et al.*, 1984; Goland *et al.*, 1986), suggesting that CRF could contribute to the increasing sensitivity to oxytocin that develops at this time.

9.2.3 PROGESTERONE

The assumption is usually made that uterine quiescence throughout human pregnancy is dependent on a direct effect of progesterone (P_4) on smooth muscle, as it does in various experimental animals. However, the supporting evidence is not conclusive, depending mainly on observations that removal of the corpus luteum (CL) before 50 days in pregnant women is followed by abortion 3 or 4 days later when the plasma P_4 has fallen from >60 to <15 nmol/l (Csapo and Pulkinnen, 1978). Administration of P_4 prevents abortion after CL excision (luteectomy). When luteectomy is performed between 50 and 60 days, P_4 concentrations fall to 30 nmol/l but pregnancy is not interrupted. Studies with pharmacological agents that inhibit P_4 synthesis or block its actions are not entirely in keeping with observed effects of luteectomy. Epostane, which interferes with the synthesis of P_4 and oestrogen by inhibiting 3β-hydroxysteroid dehydrogenase, is ineffective in inducing abortion although a sustained fall in plasma P_4 concentrations to <20% of pretreatment values is maintained for several days (Pattison *et al.*, 1984). Mifepristone (RU486), a potent P_4 antagonist that displaces P_4 from its receptor, consistently interrupts early pregnancy (Kovacs *et al.*, 1984) but is unreliable after the first trimester unless used in conjunction with a PG (Bygdeman and Swann, 1985). This difference may reflect a more complete blockade of progestational action by mifepristone or may also be due to the concomitant inhibition of oestrogen synthesis by epostane. However, neither agent reliably interrupts midtrimester pregnancy, suggesting that a reduction in the concentration or action of P_4 is insufficient in itself to cause parturition.

Low concentrations of plasma P_4 throughout pregnancy are compatible with normal-

term pregnancy (see Parker *et al.*, 1986), but administration of P_4 or potent analogues to women at term fails to prolong pregnancy (Csapo *et al.*, 1966), an observation that is not surprising in view of the likelihood that nuclear P_4 receptor sites are likely to be saturated at term and that receptor site concentrations do not diminish before labour starts (Kreitmann and Bayard, 1979). The concentration of P_4 in the myometrium increases to term and the ratio of P_4 to oestradiol-17β remains unchanged (Batra and Bengtsson, 1978).

The weight of evidence is therefore strongly against parturition at term being the result of loss of the inhibitory effects of P_4. However, P_4 may have an important role in the maintenance of human pregnancy by contributing to the maintenance of uterine quiescence.

Spontaneous and oxytocin-induced contractility of strips of human pregnant myometrium are inhibited by P_4 (Kumar *et al.*, 1962, 1967). The inhibitory effect was attributed by Daniel and Singh (1958) to hyperpolarization of smooth muscle cell membrane, but Jung (1962) and Kao and Nishiyama (1964) reported contradictory findings and the matter remains unresolved. However, P_4 has a characteristic effect on the action potential, the amplitude of which becomes irregular, with a poor correlation of action potentials and mechanical activity. Carsten (1979) reported that P_4 inhibits ATP-dependent calcium binding in a microsomal preparation of pregnant myometrium. Garfield (1988) described a marked increase in the number of gap junctions in human myometrium during labour, which is likely to cause the myometrium to function as a syncytium with enhanced propagation of action potentials. P_4 inhibits gap junction formation in rat myometrium, but evidence of such an effect in human myometrium is lacking.

Progesterone has the potential to modulate uterine contractility by influencing the concentration of various receptors. Progesterone down-regulates activity of oxytocin receptors and oestrogen receptors in rats (Soloff *et al.*, 1983). Neither of these actions is known to occur in human pregnancy, but low concentrations of oxytocin receptors until late pregnancy (Fuchs *et al.*, 1984) and of oestrogen receptors throughout pregnancy (Batra and Bengtsson, 1978) are consistent findings.

Local changes in tissue concentration of steroid hormones may take place that are not reflected in the circulation. A direct action of P_4 on the fetal membranes is unlikely because of the absence of specific binding, although specific binding of testosterone is readily demonstrable (De Cicco *et al.*, 1984). Schwarz *et al.* (1977) described a specific P_4-binding protein in amnionchorion which could regulate local concentrations of P_4. However, the protein is transcortin-like rather than a unique P_4-binding protein, having a 10-fold greater affinity for cortisol than for P_4. Decidua, like the endometrium, has specific P_4 receptors and, in view of the responsiveness to P_4 of the non-pregnant endometrium from which the decidua is derived, it is likely to be a target tissue for P_4 action in regulating oxytocin action and PG synthesis. Local P_4 concentration could also be influenced by a change in rate of metabolism at the site of its local (non-placental) production.

Challis and Mitchell (1988) suggested that P_4 acts in an autocrine or paracrine fashion and that local changes in concentration would not be detectable in the systemic circulation. The hypothesis had two main tenets: (1) that extraplacental intrauterine tissues can synthesize and metabolize P_4 and (2) that P_4 production within the fetal membranes and decidua is sufficient to not be overwhelmed by placental production or that production occurs in a compartment that is isolated from systemic influences. Confirming the report of Gibb *et al.* (1978), Challis and Mitchell (1988) demonstrated the uptake of pregnenolone (P_5) and its conversion to P_4 by dispersed cell preparations of amnion, chorion and decidua, the activity being greatest in the chor-

ion. The activity was less in tissues obtained after the spontaneous onset of labour than in those obtained at elective Caesarean section at term (B.F. Mitchell *et al.*, 1987). Amnion contains little 3β-hydroxysteroid dehydrogenase (3β-HSD), being formed predominantly from 20α-dihydroprogesterone, and activity is greatest before the onset of labour (Challis and Vaughan, 1987). Fetal plasma concentrations of pregnenolone sulphate are high in late pregnancy, indicating that the conversion of P_5 to P_4 by chorion and decidua forms an important local source of P_4 which is 'withdrawn' at the onset of labour. Pulkkinen and Enkola (1972) reported a gradient of P_4 concentrations with increasing distance from the placenta, a finding consistent with local production, but the concentration did not differ between tissues obtained before or during labour. Further support for the concept of local production of P_4 by membranes and decidua comes from the observation that the concentration of P_4 in these tissues is considerably higher than that in the maternal circulation. The role of P_4 in regulating PG synthesis is considered below.

9.2.4 OESTROGENS

The concentrations of oestrone, oestradiol-17β and oestriol rise progressively throughout pregnancy, but there is no abrupt increase before the onset of labour (Chew and Ratnam, 1976; Boroditsky *et al.*, 1978; Hartikainen-Sorri *et al.*, 1981; B.S.B. Block *et al.*, 1984) unless the subjects are highly selected (Turnbull *et al.*, 1974). The concentration of oestradiol-17β in myometrium during pregnancy is low and does not increase at term (Haukkamaa and Lahteenmaki, 1979), but an increase in the nuclear fraction in labouring patients was described by Hilary and Cohen (1981). The human placenta lacks 17α-hydroxylase activity and consequently depends on androgen (mainly dehydroepiandrosterone sulphate, DHEAS) derived from the fetal and, to a lesser extent, maternal adrenals as the substrate for oestrogen synthesis. DHEAS is present in the fetal circulation in increasing concentrations to term (Laatikainen *et al.*, 1980; Parker *et al.*, 1982).

Attempts to alter the length of pregnancy by manipulating the concentration of circulating oestrogen, either by changing the rate of production of oestrogen by the placenta or by administering oestrogen, have not been convincing. Intravenous injections of DHEAS markedly increase circulating levels of oestradiol-17β and oestriol but do not cause uterine contractions (Korda *et al.*, 1975). Conversely, administration of corticosteroids (e.g. 20 mg of prednisone per day) depresses circulating oestrogen concentrations to about 10% of normal values by inhibiting fetal and maternal adrenal secretion of DHEAS but does not prolong pregnancy. Similarly, depressed concentrations of circulating oestrogens occur in a sex-linked genetic disorder, placental steroid sulphatase deficiency, which impairs the conversion of DHEAS to DHEA (France and Liggins, 1969) but is compatible with the spontaneous onset of labour at term, although dystocia may be associated with impaired cervical ripening in some primigravid patients. Whether the administration of large doses of oestradiol-17β to women at term stimulates labour remains uncertain. Pinto *et al.* (1967) claimed that significantly more women in a controlled trial started labour within 7 days of a single intravenous dose of 200 mg of oestradiol-17β and increased uterine activity was found in uncontrolled studies after administration of large doses of oestrogens (Jarvinen *et al.*, 1965; Larson *et al.*, 1973). Flint (1979) considered that any part played by oestrogens in the onset of labour is likely to be permissive rather than obligatory.

Nevertheless, oestrogen synthesis at a local level may have an active part in the initiation of labour. The amniotic fluid contains high levels of steroid conjugates, particularly oestrone sulphate and DHEAS, both of which increase rapidly in concentration towards

term and are accessible to the fetal membranes as substrates for metabolism to oestrogens (Turnbull *et al.*, 1977). Chorion and decidua, but not amnion, contain steroid sulphatase activity capable of hydrolysing oestrone sulphate and DHEAS and liberating free oestrone and DHEA (Romano *et al.*, 1986). The presence of 17β-HSD in the membranes and decidua was evident because of the conversion of oestrone to oestradiol-17β. The greater activities of the enzymes in labour are not reflected in the tissue concentrations of oestrone or oestradiol-17β, which are the same before and after labour, and the extent to which these findings *in vitro* can be extrapolated to the situation *in vivo* is unknown.

Oestrogen administration stimulates uterine activity, but the spontaneous and oxytocin-induced contractility of myometrial strips *in vitro* is consistently inhibited by oestrogen. The inhibitory potency of oestrogen greatly exceeds that of P_4, not only in animal tissues (Hempel and Newman, 1965) but also in human tissues (Barnafi and Larraguibel, 1974). This paradox may be resolved by the stimulatory effects of oestrogen on the release of PGs which are evident *in vivo* but may be absent in muscle strips separated from the endometrium.

Oestrogen is undoubtedly important in promoting growth of both the muscular and connective tissues of the pregnant uterus, readily demonstrated by observing the response of the non-pregnant uterus to prolonged treatment with oestrogen. The uterus, although devoid of contents other than hypertrophied endometrium, may reach a size and appearance equivalent to that of pregnancy at 3 months. There is no direct evidence that oestrogen enhances oxytocin sensitivity or promotes the formation of oxytocin receptors, P_4 receptors and gap junctions in women. The role of oestrogen in the control of PG synthesis is discussed below.

9.2.5 OXYTOCIN

The discovery of oxytocin and its subsequent clinical use in human pregnancy led to observations that fulfilled many of the requirements for an agent with a dominant place in the physiological mechanism initiating parturition. Oxytocin is measurable by highly sensitive assays in most women throughout pregnancy (Kumaresan *et al.*, 1974; Dawood *et al.*, 1979a) and there is a weak trend to rising concentrations through pregnancy (Dawood *et al.*, 1979b; Sellers *et al.*, 1981). The amniotic fluid contains oxytocin, which is apparently of maternal origin because the concentration in pregnancies with anencephalic fetuses do not differ from those in women with normal fetuses (Swaab and Oosterbaan, 1983). Oxytocin is the most potent known natural substance that stimulates uterine contractions in pregnancy. During labour, spike concentrations of oxytocin may (Gibbens *et al.*, 1972; Leake *et al.*, 1981) or may not (Sellers *et al.*, 1981) occur. Intravenous infusion of oxytocin stimulates contractions at any stage of pregnancy although very large amounts are required in early pregnancy. In late pregnancy, the sensitivity to oxytocin progressively increases to the extent that a response is elicited at term by an infusion rate (1 miu/min) equivalent to the estimated rate of endogenous production (Caldeyro-Barcia and Sereno, 1961). However, oxytocin infusions are effective in inducing labour only when the pregnancy is at term or beyond, and the response is inconsistent unless amniotomy is performed concurrently. The rapid increase in oxytocin sensitivity appears to begin around the 20th week of pregnancy (Turnbull and Anderson, 1968) for reasons that are uncertain.

Although studies of oxytocin were eclipsed when the possibilities of PGs were realized, there has been a resurgence of interest in oxytocin, particularly in relation to its receptor concentrations in the myometrium and

decidua. Oxytocin may also be a specific stimulus to PG synthesis.

Crude membrane fractions of human myometrium contain specific binding sites for oxytocin that fulfil several criteria for receptors (Soloff *et al.*, 1974; Sakamoto *et al.*, 1979), including inhibition of binding of labelled oxytocin by synthetic oxytocin analogues in the rank order of their potencies. Fuchs *et al.* (1982a) obtained samples of myometrium and decidua parietalis from women at Caesarean section or hysterotomy at or before term. Crude membrane fractions were prepared from homogenates incubated with EDTA to dissociate endogenous oxytocin from binding sites. Myometrial receptor concentrations increased eightfold from low concentrations at 13–17 weeks to term and more than doubled early in labour. In advanced labour, the concentration had fallen sharply, possibly because the samples taken from lower segment incisions were composed mainly of cervical tissue. Similar concentrations of receptor sites and changes with gestation length and labour were found in decidua. It was suggested that the increase in receptors lowers the sensitivity of the myometrium to oxytocin to the point where activation occurs and labour begins.

Incubation of decidua parietalis with a high concentration of oxytocin stimulates the release of PGE and PGF (Fuchs *et al.*, 1981; T. Wilson *et al.*, 1988), suggesting that myometrial activity is stimulated by oxytocin directly and indirectly by PG liberated from the decidua.

The increase in receptors with the onset of labour in women cannot be attributed to an increase in the oestrogen–P_4 ratio, which appears to be unchanged. In rats there is a close correlation between the concentrations of oxytocin receptors and tissue $PGF_{2\alpha}$ and a marked reduction in receptors during treatment with an inhibitor of PG synthesis (W.Y. Chan, 1987). Enhanced responsiveness to oxytocin after treatment with PGE_2 occurs in human pregnancies near term (Embrey, 1969; Brummer, 1971) and *in vitro* in excised human myometrial strips (Brummer, 1971) and is consistent with the formation of receptors in response to PGs.

An oxytocin antagonist, 1-deamino-2-D-Tyr(OE+)-4-Thr-8-Orn-oxytocin (CAP 440), inhibits oxytocin-stimulated activity of human myometrial strips (Melin *et al.*, 1986), and appeared to inhibit contractions in preterm labour in an uncontrolled pilot study (Akerlund *et al.*, 1987).

Chibbar *et al.* (1991) demonstrated the presence and expression of oxytocin mRNA in chorionic membrane and decidua and concluded that oxytocin is synthesized in fetal membranes. Whether the rate of synthesis changes at the onset of labour remains to be determined.

9.2.6 RELAXIN

Human relaxin, a protein of approximately 6 kDa molecular weight, has been sequenced (Hudson *et al.*, 1983) and recombinant material is available. It shares considerable structural homology with insulin and the insulin-like growth factors. Despite progress with genetic aspects of human relaxin, the role of relaxin on the myometrium in human pregnancy remains enigmatic (for review see Bryant-Greenwood, 1991).

Plasma concentrations of relaxin in pregnant women are low compared with sows (Eddie *et al.*, 1986) and fall progressively through pregnancy, showing no evidence of the marked prepartum rise in concentration that is characteristic of certain animal species. In early pregnancy relaxin is secreted by the CL and circulating concentrations become undetectable after luteectomy. Whether other sources, such as the decidua, contribute to plasma levels in late pregnancy is unknown.

Immunofluorescence studies using antibodies raised against the connecting peptide of human relaxin (Sakbun *et al.*, 1987) have demonstrated the presence of relaxin in the decidua at term, and it is likely that

the tissues synthesize rather than sequester the hormone because it persists after luteectomy (Yki-Jarvinen and Wahlstrom, 1984). Although the concentration of relaxin in decidua is very low, Bryant-Greenwood and Greenwood (1988) postulated that the hormone diffuses from the site of production to adjacent smooth muscle and connective tissues to exert a variety of biological actions.

Porcine relaxin applied to the cervix of women at term causes it to ripen rapidly in a manner similar to that occurring normally before labour or after treatment with PGs (MacLennan *et al.*, 1980), but whether endogenous relaxin promotes collagenolysis in the cervix and fetal membranes under physiological conditions remains to be determined. The effect of relaxin is uncertain (Beck *et al.*, 1982; MacLennan *et al.*, 1986). Indeed, a report that relaxin inhibits the production of PGE by amnion when the membrane is taken at elective Caesarean section, but stimulated production in membranes taken after spontaneous labour (Lopez-Bernal *et al.*, 1987a), suggests that inhibition or stimulation of uterine activity might occur, depending on the circumstances.

9.2.7 PROLACTIN

Prolactin, produced by the decidua, accumulates in high concentrations in amniotic fluid, reaching maximal values at 14–18 weeks. Although placental prolactin is immunologically indistinguishable from pituitary prolactin and is biologically active, control of its secretion appears to be independent of the dopamine system (Golander *et al.*, 1979) but dependent on a product of arachidonic acid metabolism, its secretion being inhibited by arachidonic acid (Handwerger *et al.*, 1981). Decidual prolactin is probably involved in the regulation of water exchange across the fetal membranes and the control of amniotic fluid volume (see McCoshen, 1988 for review) but the relevance of decidual prolactin to parturition is unclear. Krug *et al.* (1983) reported that the production rate by decidua obtained after spontaneous labour was greater than that obtained at elective Caesarean section, which is inconsistent with the observation that prolactin inhibits the production of PGE in incubates of combined amnion–chorion–decidua (Tyson *et al.*, 1985). Furthermore, the finding that steroid sulphatase activity in decidual cells harvested at delivery is higher than in those harvested at elective Caesarean section (Braverman and Gurpide, 1986) is inconsistent with the observation that prolactin stimulates the hydrolysis of oestrone sulphate (sulphatase activity) in prelabour cells, whereas hydrolysis in cells obtained after delivery is inhibited (Krug *et al.*, 1983). Further investigations are required to elucidate the interrelationships of decidual prolactin, hydrolysis of oestrogen sulphates and synthesis of PGs.

9.2.8 EICOSANOIDS

The evidence that eicosanoids dominate the mechanism initiating human labour is strong but not conclusive. A crucial piece of evidence (the release of eicosanoids immediately preceding the start of labour) is missing, which is not unexpected when the difficulties of predicting labour and of obtaining samples from suitable sites are considered. The hypothesis that favours involvement of eicosanoids is supported by a number of clinical observations.

1. Administration of prostaglandin (PG)$F_{2\alpha}$, E_2 or various active analogues causes abortion or labour at any stage of pregnancy from soon after implantation to term. The clinical features of abortion or labour mimic those of the spontaneous event and include not only myometrial contractions but also increased distensibility and effacement of the cervix. Eicosanoids differ in this respect from other oxytocic agents such as oxytocin, which cause strong contractions but not cervical effacement or

progressive labour unless the pregnancy is near term.
2. PG antagonists such as indomethacin or fenamic acid derivatives inhibit labour even when the cervix is partly dilated. Labour resumes when the antagonist is withdrawn.
3. The concentrations of $PGF_{2\alpha}$, PGE_2 and their metabolites in amniotic fluid and plasma increase progressively after the onset of labour.
4. Successful, but not unsuccessful, induction of labour at term with oxytocin is associated with a sustained increase in the concentration of prostaglandin F metabolite (PGFM) in maternal plasma.
5. Infection (amnionitis) and trauma (amniotomy), which cause release of eicosanoids, are often followed by labour.
6. Intra-amniotic injection of arachidonic acid causes abortion.
7. No agents are known to induce abortion or labour without having at least the potential for stimulating release of eicosanoids.

Measurements of primary eicosanoids in tissues and fluids that accurately reflect concentrations *in vivo* are difficult to obtain for a number of reasons. In tissues, they are rapidly released rather than stored and so the concentrations are very low. Trauma stimulates the rapid synthesis of arachidonic acid metabolites, which then accumulate during the short time between excising and processing tissues. The primary PGs, particularly PGE_2, are unstable and tend to decompose during extraction. Furthermore, PGE_2 and thromboxane (TX) A_2 are released by platelets trapped in tissues and present in blood samples. These problems have been overcome to a large extent by the addition of enzyme inhibitors such as aspirin to collected samples and by assays of stable metabolites such as PGFM, bicyclo-PGE, TXB_2 and 6-keto-PGF_1. Stabilizing primary PGs by methoximating them in the sample and assaying the conjugate with a specific antibody is an alternative approach that is proving successful.

(a) Concentrations in plasma and amniotic fluids

Reports of plasma concentrations of $PGF_{2\alpha}$ in pregnancy are notable mainly for lack of agreement on absolute values or patterns. In reviewing these reports, Keirse (1979) concluded that the concentrations of PGF in the peripheral circulation cannot be interpreted as a measure of uterine PG production. Assays of PGFM, however, have resulted in more consistent results showing low and unchanging values throughout pregnancy with a marked rise in established labour and a progressive increase as the cervix dilates (Green *et al.*, 1974; Fuchs *et al.*, 1983a; Nagata *et al.*, 1987). Close sequential sampling immediately before and after labour has not been reported, and whether PGFM values rise before labour is unknown. Rasmussen *et al.* (1985) found up to a 25-fold increase in concentrations of PGFM in peripheral plasma in women infused at term with $PGF_{2\alpha}$ at a rate sufficient to induce labour, a finding they interpreted as indicating that if $PGF_{2\alpha}$ is causally related to the onset of spontaneous labour generation must take place locally in tissues in, or close to, the site of action. The concentrations of PGF and PGFM in amniotic fluid follow a pattern similar to that of PGFM in plasma, showing no changes before the onset of labour but a progressive increase during labour (Keirse, 1979). Amniotomy is associated with a rapid rise in concentration of PGFM and TXB_2 but not of 6-keto-$PGF_{1\alpha}$ in amniotic fluid (M.D. Mitchell *et al.*, 1978a, 1979a). Makarainen and Ylikorkala (1984) confirmed the increase in TXB_2 but found that levels of 6-keto-PGF_1 also increased. The observation that the concentrations of PGFM in plasma and amniotic fluid rise early during spontaneous labour but not until late in oxytocin-induced labour (Keirse *et al.*, 1974;

Fuchs *et al.*, 1983a) is consistent with the idea that the release of $PGF_{2\alpha}$ is causally related to the onset of labour rather than being a consequence of uterine contractions.

The concentration of PGE_2 in amniotic fluid is similar to that of PGF_2 and rises in parallel (Keirse and Turnbull, 1972), but there is disagreement about plasma values. M.D. Mitchell *et al.* (1978b), who assayed PGE, found no significant change in PGE, whereas Husslein (1984) found an approximate doubling of PGE metabolite (PGEM) values in labour. Assays of bicyclo-PGEM (a very stable substance) showed little (M.D. Mitchell *et al.*, 1982a) or no (Brennecke *et al.*, 1985) increase in plasma concentration in labour.

Products of the lipoxygenase pathway are present in amniotic fluid and the concentrations are higher in labour. Concentrations of 12-HETE (hydroxyeicosatetranoic acid) are similar to those of PGE_2 and $PGF_{2\alpha}$, whereas those of 15-HETE and leukotriene $(LT)B_4$ are much lower (Romero *et al.*, 1987a).

Measurements of eicosanoids in tissue are unlikely to indicate concentrations *in vivo*, but probably indicate the capacity of the tissue for synthesis which, assuming massive release of arachidonic acid in response to trauma, is likely to be rate-limited by PGH synthase. With these qualifications, the data of Willman and Collins (1976) indicate that the amnion and decidua have the highest concentrations after labour, which is consistent with observations of production rates of incubated or perfused tissues.

(b) Production of eicosanoids by intrauterine tissues

Eicosanoids modulate cell activities throughout the body, but the tissues of the uterus and fetoplacental membranes show considerable variation in the quantity of eicosanoids released and in the spectrum of products. Production rates have been studied in several ways, including measurement of radioactive products formed from labelled arachidonic acid and measurement of unlabelled materials using static incubations of homogenates, organ cultures or superfusion systems. Organ culture or superfused dispersed cells are the preferred systems. In general, results with different systems are similar. The amnion is the most active tissue and generates predominantly PGE_2 and lesser amounts of $PGF_{2\alpha}$ (M.D. Mitchell *et al.*, 1978c; Okazaki *et al.*, 1981a; Olson *et al.*, 1983; Manzai and Liggins, 1984; K.A. Skinner and Challis, 1985). Myatt *et al.* (1985) found that radiolabelled confluent cultures of amnion cells release mainly HETEs when the cells are obtained before labour and mainly PGE_2 when the cells are obtained during labour.

Decidual tissue releases more $PGF_{2\alpha}$ than PGE_2 or PGI_2 (Olson *et al.*, 1983, Manzai and Liggins, 1984), whereas release of PGI_2 predominates in cultures of myometrium (Omini *et al.*, 1979). The source of PGI_2 in myometrium is likely to be vascular endothelium, but smooth muscle cells also produce PGI_2 (Keirse *et al.*, 1984). The chorion cells release $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ but are most active in the metabolism of prostanoids by 15-dehydrogenase and reductase enzymes (Okazaki *et al.*, 1981b), which is reflected in a greater output of PGFM than from other tissues (K.A. Skinner and Challis, 1985). Comparison of the rates of production of prostanoids by decidua and fetal membranes obtained before or after the onset of labour consistently shows greater activity of the in-labour tissues (Olson *et al.*, 1983), but the cause is uncertain (see below). The specific activity of phospholipase (PL) C and PLA_2 measured in the various tissues is unchanged with the onset of labour (Okazaki *et al.*, 1981a), but this may reflect the difficulty of assessing the activity *in vitro* of an enzyme such as PLA_2 that is present *in vivo* mainly in an inactive form but readily activated by extraction procedures.

Other sources that may contribute to the pool of prostanoids in amniotic fluid include

the umbilical cord (cultures of which release PGI_2 from the blood vessels), the amniotic epithelium, predominantly PGE_2 (M.D. Mitchell *et al.*, 1980a), and fetal urine, which contains PGE_2 and $PGF_{2\alpha}$ in appreciable concentrations (Casey *et al.*, 1983a).

The hypothesis that PGE_2 arising from the amnion initiates labour has been suggested because the major prostanoid formed by fetal membranes *in vitro* is PGE_2, the concentration of PGE_2 in amniotic fluid rises in labour, the rate of production of PGE_2 increases in labour and PGE_2 is more potent than $PGF_{2\alpha}$ in inducing parturition. However, more reliable assays of PGE_2 metabolites reveal no increase in circulating concentrations during labour (Brennecke *et al.*, 1985). Moreover, doubt has arisen as to the amount of unmetabolized PGE_2 that is able to pass through the membranes to reach the decidua and myometrium. McCoshen *et al.* (1987) studied membranes mounted in a dual-chamber perfusion apparatus which allowed the passage of labelled PGE_2 in either direction: about 50% of PGE_2 placed on the fetal side of the amnion alone crossed the membrane within 4 h and less than 5% traversed the combined amnion and chorion. To the contrary however, Nakla *et al.* (1986) reported that unmetabolized PGE_2 traversed full-thickness membranes in either direction and the rate of passage was greater after labour. The reason for these disparate results is not clear. Furthermore, pregnancies in which the amnion is detached from the chorion deliver normally at term unless the membranes rupture prematurely.

Casey and MacDonald (1988) reject amnion as the primary source of PGs, preferring the decidua as the source and $PGF_{2\alpha}$ rather than PGE_2 as the active PG for a number of reasons. They argue that through most of pregnancy the rate of production of $PGF_{2\alpha}$ by the decidua is rate limited by inhibition imposed by the fetal decidual paracrine system and that it is withdrawal of this inhibition that leads to labour. The amnion acquires arachidonic acid only from the amniotic fluid and, although arachidonic acid is lost from the membranes in early labour, the content is restored in late labour at a time when the concentration of arachidonic acid in amniotic fluid is rising rapidly (Keirse *et al.*, 1977; Okita *et al.*, 1983). This suggests the decidua as a source of arachidonic acid entering the membranes and amniotic fluid.

Casey and MacDonald (1988) calculated that the rates of production of $PGF_{2\alpha}$ and PGE_2 are low, even in labour, and that because of the very high rate of clearance in the lungs the concentrations in maternal peripheral blood may not increase measurably. They compared the rate of intravenous infusion of $PGF_{2\alpha}$ needed to induce labour with estimates of the rate at which endogenous $PGF_{2\alpha}$ is released (calculated from the plasma concentration of PGFM and the rate of its clearance). The rate of uterine secretion of $PGF_{2\alpha}$ estimated in this way is about 100 ng/min, and the rate at which infused $PGF_{2\alpha}$ reaches the uterus is about half this value. Although these approximations involve a number of assumptions, it appears that sufficient $PGF_{2\alpha}$ is released in early labour to be responsible for its onset. Whether the source of $PGF_{2\alpha}$ is decidua or myometrium is uncertain, but decidua is favoured because levels of 6-keto-$PGF_{1\alpha}$ in maternal plasma or amniotic fluid do not rise in parallel with PGFM, as would be expected if myometrium was the source. The possibility that decidual PGF_2 arises from amniotic PGE_2 converted by the enzyme 9-keto-reductase can be ruled out because the specific activity of the enzyme in decidua is much lower than that of 15-keto-PG dehydrogenase, which would result in inactivation of PGE_2 rather than conversion to $PGF_{2\alpha}$ (Niesert *et al.*, 1986).

The decidual stromal cells in which phospholipids are highly enriched with arachidonic acid are generally considered to be the cell type mainly responsible for the synthesis of $PGF_{2\alpha}$, although epithelial cells also have this capacity. Decidual stromal cells may be

macrophage-like, derived in part from bone marrow cells (Bulmer and Sutherland, 1983; Casey and MacDonald, 1988), because both are sites of 1-hydroxylation of 25-OH-vitamin D_3 and of production of β-endorphin and both respond to exposure to bacterial endotoxin by releasing quantities of PGs and cytokines. An inconsistency is the predominant production of $PGF_{2\alpha}$ by decidua but of PGE_2 by macrophages. More than one source and more than one prostanoid may be involved in the initiation of labour; for example, amniotic PGE_2 may enhance formation of various receptors in decidua and decidual $PGF_{2\alpha}$ may activate the myometrium.

(c) Control of PG synthesis

Evidence for many aspects of the system regulating PGs in pregnant human uterine tissues is missing or uncertain, making a coherent description impossible. To provide some orderliness to the fragments that are available, the following review will refer each section to Figure 9.1, with an appended numeral referring to a numeral on the diagram, i.e. Figure 9.1.1, etc.

Arachidonic acid is stored in fetal membranes and decidua (Figure 9.1.1), mainly in the *sn*-2 position of the glycerophospholipids, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, all of

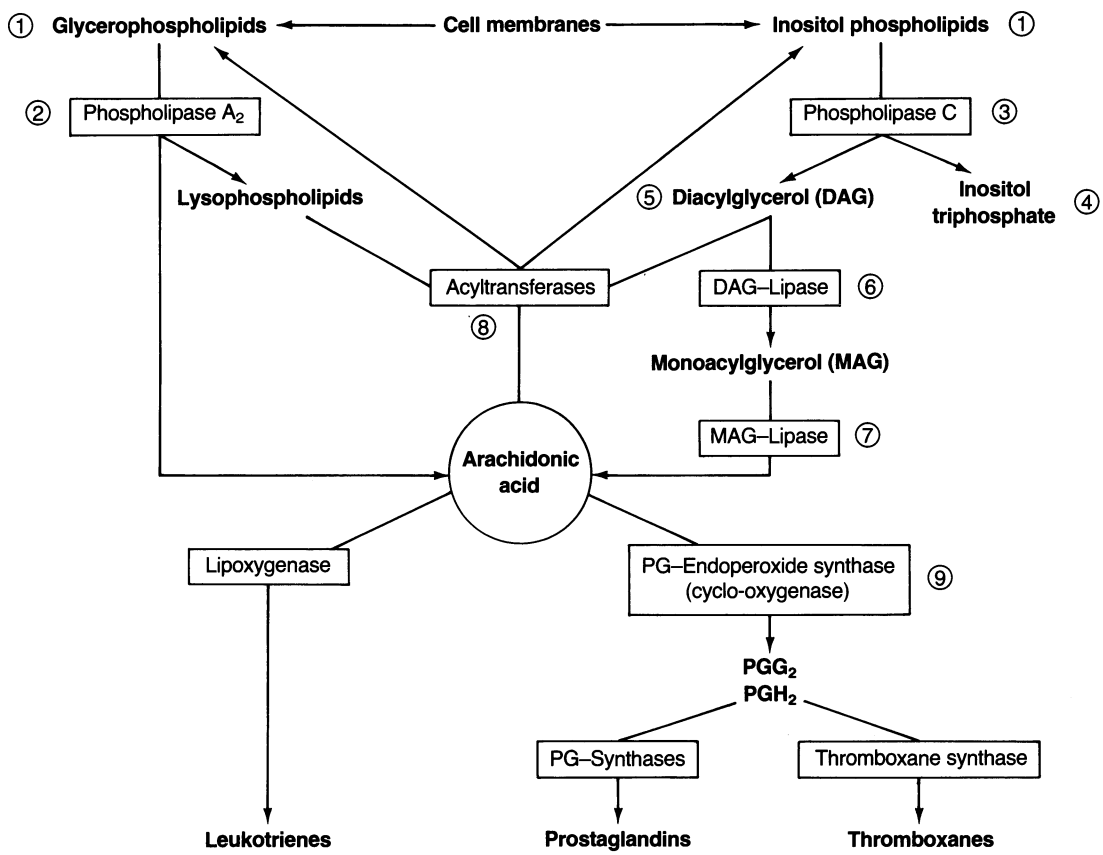

Figure 9.1 The arachidonic acid cascade. See text for details.

which are enriched with arachidonic acid relative to other tissues (Okita *et al.*, 1982a) for reasons that are uncertain but possibly due to an effect of P_4. Triglycerols in the membranes are not enriched and do not become depleted of arachidonic acid in labour. The release of arachidonic acid is controlled by one of two phospholipases. Many studies of PL activity have been in fetal membranes and there is a dearth of information about decidua. Phospholipase A_2 activity (Figure 9.1.2) in fetal membranes is relatively specific for phosphatidylethanolamine substrates (Okazaki *et al.*, 1978) and is a microsomal enzyme that is activated by Ca^{2+} at neutral pH, unlike lysosomal PLA_2, which is activated at acid pH independently of Ca^{2+}. The rate of release of arachidonic acid from 1-palmitoyl, 2-arachidonyl glycerophosphoethanolamine by PLA_2 is approximately four times that from the corresponding form of phosphatidylcholine. In addition, PLA_2 has specificity for glycerophospholipids containing arachidonic acid rather than oleic acid at the *sn*-2 position. Phospholipase C (PLC), which has specificity for phosphatidylinositol (Figure 9.1.3), is the first in a series of enzymes that release arachidonic acid from amnion and is Ca^{2+} dependent (DiRenzo *et al.*, 1981). Phospholipase C effects the release of inositol triphosphate (IP_3) (Figure 9.1.4), giving rise to diacylglycerol (DAG) (Figure 9.1.5), which becomes the substrate for DAG diacylglycerol lipase (Figure 9.1.6) with liberation of the fatty acid from the *sn*-1 position. A monoacylglycerol lipase (Figure 9.1.7) catalyses the final step, liberating arachidonic acid from the *sn*-2 position. DAG lipase and monoacylglycerol lipase are present in fetal membranes and decidua (Okazaki *et al.*, 1981c). The concentration of free arachidonic acid is determined not only by the activity of the PLs and the rate at which it is metabolized but also by the rate at which it is reincorporated into glycerophospholipids by lysophosphatide acyltransferase activity (Figure 9.1.8) and at which DAG is recycled through diacylglycerol kinase activity to phosphatidic acid and thence back to phosphatidylinositol. The increase in the concentration of DAG observed in in-labour amnion (Okita *et al.*, 1982b) is consistent with activation of the PLC pathway during labour and with the finding that the specific activity of DAG lipase in amnion, chorion and decidua does not increase from early to late pregnancy (Okazaki *et al.*, 1981a), suggesting that the production of arachidonic acid through the PLC pathway may be limited to some extent by DAG lipase activity. However, the relative importance of PLC-mediated and PLA_2 -mediated release of arachidonic acid as substrate for PG synthesis in labour is unknown. Prostaglandin synthesis may be initiated by agonist-activated PLC activity, leading to subsequent liberation of arachidonic acid from DAG, and the secondary release of PGs then results from activation of PLA_2 by-products (DAG and IP_3) of the PLC pathway, but studies of human uterine tissues to assess such a hypothesis are lacking.

In general, PG production is rate limited by arachidonic acid availability determined by PL activity, but some evidence in animal species suggests that PGH synthase (PGHS) (Figure 9.1) may also impose restraint on the rate at which arachidonic acid is metabolized to prostanoids in intrauterine tissues (J.S. Robinson *et al.*, 1978; Risbridger *et al.*, 1985). In women, the concentration of PGHS in placental and myometrial microsomes (Keirse *et al.*, 1984) increases threefold in pregnancy, which is consistent with the enzyme being rate limiting in early pregnancy. At term, however, the extremely rapid release of $PGF_{2\alpha}$ into the maternal circulation and amniotic fluid after amniotomy or cervical stimulation (Turnbull *et al.*, 1977) does not suggest low capacity for metabolism of arachidonic acid. *In vitro* experiments in which arachidonic acid is added to homogenized tissues or cell cultures are difficult to interpret because the release of endogenous arachidonic acid may already be high. Even so, prep-

arations of chorionic membrane and decidua *in vitro* respond to the addition of arachidonic acid with increased production of PGE_2 (Lopez-Bernal *et al.*, 1987b). Present evidence points to the rate-limiting step in prostanoid synthesis at term as being phospholipase activity, but this does not exclude PGHS activity as a limiting factor in earlier pregnancy. Indeed, Marshall *et al.* (1987) estimated that the capacity of the human body for the synthesis of PGs is 1000 times greater than the amount actually produced (as measured by urinary excretion), and they argued that the biosynthetic system must be restrained under normal circumstances by factors in addition to availability of arachidonic acid. They postulate the natural self-destruction of PGHS and the destruction by peroxidases of the hydroperoxides formed by the enzyme on which its activity is dependent as two of these factors. Presumably, PGHS activity places a ceiling on the magnitude of the response in PG production that can be elicited by arachidonic acid release.

The activity of PGHS may be subject to regulation by protein kinase C. Phorbol esters and synthetic DAG, both of which stimulate protein kinase C, also stimulate PGHS in dispersed amnion, and this action as well as that of epidermal growth factor (EGF) and glucocorticoids is blocked by staurosporine, an inhibitor of protein kinase C (Zakar and Olson, 1988, 1989).

(d) Stimuli of prostanoid synthesis

Various agents, of maternal and fetal origin, stimulate arachidonic acid release and are thought to activate PLs, but little detail is available from studies of human tissues. A role for oxytocin in stimulating PG synthesis is supported by clinical and biochemical observations. Oxytocin receptors in the myometrium and decidua increase with the start of labour. Infusion of oxytocin to induce labour in women at term is associated with a sustained increase in concentrations of PGFM in the circulation of those in whom the infusion is successful but a transient increase only in those in whom labour does not become established (Fuchs *et al.*, 1983a). The decidua or the myometrium is the likely source of the PG release in response to oxytocin because the response persists for at least 2 h after delivery of the placenta and membranes (Fuchs *et al.*, 1982b). Oxytocin stimulates release of PGE and $PGF_{2\alpha}$ from incubated portions of term decidua but not from myometrium (Fuchs *et al.*, 1981). Perfusion of a dispersed decidual cell preparation pulse labelled with [^{3}H]-arachidonic acid showed that oxytocin (and histamine and bradykinin) stimulates an immediate release of arachidonic acid and PGF_2 which is greater from cells obtained after labour than before labour (T. Wilson *et al.*, 1988). The response to oxytocin is presumably mediated by oxytocin receptors, and certain post-receptor responses in myometrial and decidual cells have been described by Schrey *et al.* (1987): oxytocin and vasopressin stimulated the accumulation of inositol phosphates (Figure 9.1.4) and DAG (Figure 9.1.5), thereby demonstrating activation of PLC. Both the binding of oxytocin to its receptor and the release of inositol phosphates by hydrolysis of phosphatidylinositol are inhibited by the oxytocin antagonist CAP 440 (Lopez Bernal *et al.*, 1989). Guanosine triphosphate-binding proteins (G proteins) that are thought to transduce receptor-mediated signals have yet to be studied in uterine tissues.

The roles of oestrogen and P_4 in stimulating prostanoid release in human pregnancy are indeterminate because of the difficulty in manipulating hormonal actions *in vivo*. The P_4 receptor antagonist, mifepristone, is relatively ineffective in inducing mid-term labour unless supplemented by PG (Baulieu *et al.*, 1987), and this suggests that P_4 withdrawal may not be involved in promoting PG release. Production of PGs is increased slightly but not significantly in decidual cell cultures in response to P_4 and

mifepristone compared with that to P_4 alone (Kelly and Smith, 1987).

Oestradiol-17β stimulates the production of $PGF_{2\alpha}$ by incubated dispersed cell preparations of non-pregnant endometrium (S.J.M. Skinner *et al.*, 1984; Schatz *et al.*, 1987) and by endometrial explants (Leaver and Richmond, 1984), but studies of tissues from pregnant women have not been reported.

Amniotic fluid contains a number of constituents that stimulate prostanoid production, but most remain uncharacterized and the site of action undetermined. Platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is known to stimulate the synthesis of prostanoids in various tissues (Hanahan, 1986). Amniotic fluid from women before the onset of labour contains no measurable PAF, but amniotic fluid from about half a group of women in labour contained PAF activity detected by chromatography and platelet aggregation (Billah and Johnston, 1984). Similar findings were reported by Nishihira *et al.* (1984), who identified PAF by mass spectrometry. PAF stimulates contractions of human myometrium *in vitro* (Tetta *et al.*, 1986), and this effect is thought to be caused by an increase in cytosolic Ca^{2+}. Several possible sources of PAF have been suggested. PAF is detectable in the urine of newborns (Billah and Johnston, 1984) and is formed in organ cultures of fetal lung and may enter the tracheal fluid to reach the amniotic fluid (D.R. Hoffman *et al.*, 1986). Amnion contains PAF and its precursors and has the capacity to synthesize PAF, but Billah *et al.* (1985) were unable to demonstrate its release. The production of PGE_2 by amnion is stimulated by PAF, probably by increasing cytosolic Ca^{2+} and activating PLC and PLA_2. Angle *et al.* (1988) proposed that the release of prostanoids and the onset of labour is stimulated by PAF entering amniotic fluid from the maturing lung, but the normal duration of pregnancy in association with fetuses with complete tracheal atresia (which prevents the passage of lung fluid) weighs against this hypothesis. A similar hypothesis was proposed by Lopez-Bernal *et al.* (1988), who showed that the production of PGE by incubated discs of amnion was stimulated by human pulmonary surfactant. The fatty acids of surfactant glycerophospholipids are composed of about 2% arachidonic acid, and surfactant may provide substrate for prostanoid synthesis.

Casey *et al.* (1983b) identified a protein or protein-associated substance in fetal urine that markedly stimulated PGE_2 synthesis in amnion cells in monolayer culture and suggested that it was of renal origin. Strickland *et al.* (1983) showed that unextracted fetal urine in a concentration of 20% or greater stimulates PGE_2 synthesis by bovine seminal vesicle microsomes. The activity was heat stable and of molecular weight <12 kDa but was not purified. Hypotheses proposing a fetal urinary constituent as a trigger to labour are not supported by experiments of nature since fetuses that are unable to urinate because of lack of kidneys or obstruction of the renal tract are delivered spontaneously at term.

A fraction of amniotic fluid which passes through a membrane with a molecular weight exclusion of 1000 stimulates PGE_2 production by bovine seminal vesicle microsomes (Cohen *et al.*, 1985). The stimulatory activity increases throughout pregnancy and is greatest in labour. The relationship of this activity to the activity described in fetal urine is unclear, but both are of low molecular weight. Rehnstrom *et al.* (1983) demonstrated that crude amniotic fluid from late pregnancies in an unspecified concentration increases production of PGE and PGF by decidua and myometrium. The possibility that the active material is arachidonic acid was not excluded.

Schrey *et al.* (1987) used the ability of the phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA) to bind to and activate protein kinase C (thereby mimicking the action of

DAG) to explore the role of DAG in stimulating prostanoid production in decidual cells. TPA rapidly stimulated release of arachidonic acid from phosphatidylcholine, accompanied by a fall in phosphatidylcholine, extracellular accumulation of choline and cellular accumulation of phosphorylcholine. Schrey *et al.* (1987) interpret their observations as consistent with a model in which protein kinase C activation leads to arachidonic acid mobilization from decidual cell phospholipid by a mechanism involving PLA_2 -mediated phosphatidylcholine hydrolysis and PLC-mediated phosphatidylinositol hydrolysis, coupled with further hydrolysis of the DAG product. This study incidentally raises the possibility that decidua differs from amnion in that phosphatidylcholine rather than phosphatidylethanolamine may be the major source of arachidonic acid for metabolism to prostanoids. Schrey *et al.* (1987) did not suggest a mechanism linking protein kinase C with activation of phospholipases but it may inactivate a phospholipase inhibitory protein.

EGF, which is present in amniotic fluid, stimulates the production of PGE_2 and lipoxygenase products by confluent cultures of amnion cells (M.D. Mitchell, 1987), but there is no evidence that the concentration of EGF or its receptor undergoes changes at term. Amnion discs release PGE_2 into the incubation medium when exposed to low concentrations of isoproterenol (DiRenzo *et al.*, 1984). The response is inhibited by propanolol and reproduced by dibutyryl cyclic AMP, which supports beta-adrenergic stimulation.

Various non-physiological agents presumed to stimulate PG release are used as methods of inducing abortion or labour or arise as a consequence of pathological states in pregnancy. They include intra-amniotic hypertonic or irritant solutions, extra-amniotic fluids or foreign bodies and amnionitis. The concentration of $PGF_{2\alpha}$ in amniotic fluid is raised 24 h after the intra-amniotic injection of hypertonic saline when uterine activity is beginning (Gustavii and Greén, 1972). The effects of amnionitis, which is an important cause of preterm labour, have been studied extensively. Bacterial endotoxin (a lipopolysaccharide), released into the amniotic fluid in substantial quantities during infection (Romero *et al.*, 1987b), stimulates the production of cytokines by decidua (Romero *et al.*, 1989a–c). Three of these cytokines, interleukin 1α, interleukin 1β and tumour necrosis factor, which accumulate in amniotic fluid, are potent stimuli of $PGF_{2\alpha}$ by decidua (Casey and MacDonald, 1988; M.D. Mitchell *et al.*, 1990) and are thought to mediate the effect of amnionitis in provoking preterm labour. The mechanism by which cytokines stimulate prostanoid synthesis in decidua has not been studied.

(e) Inhibitors of prostanoid synthesis

Increased prostanoid synthesis at term could reflect either the activity of various stimuli, the withdrawal of tonic inhibition or both of these. Several putative inhibitors are present in pregnancy, but evidence of a reduction in effect at term is lacking for most of them.

Abel *et al.* (1980) measured the concentration of PGE and $PGF_{2\alpha}$ in the endometrium of women with intrauterine or ectopic (tubal) pregnancies and found that the concentrations are significantly less than in the endometrium throughout the normal menstrual cycle. Because the effect on the endometrium is the same whether the conceptus is inside or outside the uterus, Abel *et al.* (1980) concluded that the suppression of endometrial synthesis of PGs in early pregnancy may be mediated systemically rather than through a local action of the conceptus. The obvious candidate for a circulating inhibitor is P_4, which reduces the production of $PGF_{2\alpha}$ by secretory endometrium in organ cultures over a period of days (Kelly and Smith, 1987). However, the concentration of PGs is raised in mid-luteal endometrium, when P_4 levels are as high as in early preg-

nancy (Levitt *et al.*, 1975; Mathius and Kelly, 1978), and the production rate of $PGF_{2\alpha}$ in perfused endometrium is maximal in the late luteal phase (S.J.M. Skinner *et al.*, 1984). The apparent conflict between the known inhibitory effect of P_4 and the high concentration of PGs present in secretory endometrium is probably reconciled by reports from animal studies showing that although P_4 inhibits synthesis it also increases the capacity for synthesis *in vivo* (Kelly and Smith, 1987). The concentration of PGs in excised tissues probably reflects the capacity for synthesis rather than the actual levels present *in vivo* because of rapid production by the traumatized tissue. The contrast of the concentrations in endometrial tissue in the mid-luteal phase (which are high) with the production rates in perfused tissues at the same time in the menstrual cycle (which are low) (Liggins *et al.*, 1980), suggests that the capacity for synthesis of PGs is impaired in early pregnancy by a circulating agent that is unlikely to be P_4. The addition of 10^{-6} M progesterone to dispersed decidual cells reduces the rate of release of arachidonic acid within 15 min, although it has no effect on PLA_2 activity in a cell-free system (T. Wilson *et al.*, 1986), suggesting a direct physical action rather than an effect mediated by nuclear receptors and transcription of mRNA. A similarly rapid effect of P_4 on contractions of the monkey vas deferens stimulated by Ca^{2+} was noted by Moroshita (1986).

Various unidentified compounds with inhibitory effects on PG synthesis have been located in amniotic fluid or incubation media conditioned by decidua or amnion. Saeed *et al.* (1982) studied the effect of crude amniotic fluid on the rate of conversion of arachidonic acid to PGE_2 by bovine seminal vesicle microsomes and found that inhibitory activity is greatest in early pregnancy, is less at term and falls further in labour. The inhibitory activity is directed against PG synthase and may be proteinaceous (Brennecke *et al.*, 1983). A preliminary report by Reddi and Norman (1985) described inhibition of microsomal PGE and PGF production by amniotic fluid from about one-third of women not in labour, but PGF was not inhibited during labour. Culture medium conditioned by decidua from women at term but not in labour has marked inhibitory effects on the production of PGE_2 by amnion cells in monolayer culture (Romero *et al.*, 1987c), but Manzai and Liggins (1984) reported inhibition of endometrial production of PGE and PGF by medium conditioned by incubation with dispersed amnion cells from non-labouring women: the inhibitory action was absent when amnion cells from women in labour were used. The inhibitory effect is not reversed by arachidonic acid, suggesting that the site of action may be on PG synthase.

An endogenous inhibitor of PG synthase (EIPS) identified in plasma of human and other animals was postulated as playing a part in parturition (Saeed *et al.*, 1977), but measurements of plasma concentrations of EIPS by radioimmunoassay throughout pregnancy and labour show no trends consistent with lessening of inhibitory activity with the onset of labour (Brennecke *et al.*, 1984). A protein known as pregnancy-associated prostaglandin synthase inhibitor (PAPSI) was identified in human amniotic epithelium by immunohistochemistry using an uncharacterized antibody against PAPSI (Mortimer *et al.*, 1985); although the protein was present in most of the tissues of 15 women at term, none was found in five women in labour.

Recent interest has centred on proteins with PLA_2 inhibitory activity belonging to the lipocortin family, which were first identified by Hirata *et al.* (1980) and Blackwell *et al.* (1980). The glucocorticoid-dependent lipocortins have molecular weight of 15–37 kDa. They are polar glycoproteins containing about 10% carbohydrate. Lipocortin I (M_r 37 000) has been sequenced and cloned (Wallner *et al.*, 1986) and has partial homology with kinases and their substrates. Lipocortin mRNA is present in large amounts

in lung, thymus, spleen and placenta. Short stretches of internal hydrophobic residues may be involved in binding Ca^{2+}. Analysis of genomic DNA reveals only one unique gene, which suggests that other PLA_2-inhibitory proteins in the same family are products of proteolytic cleavage or of aggregation. The physiological role of lipocortins I and II (M_r 37 000) is not known, and it is possible that PLA_2 inhibitory activity is not their main function. The lipocortin molecule is inactivated by phosphorylation (Hirata, 1983), which is probably catalysed by protein kinase C because activation of protein kinase C by phorbol ester stimulates PLA_2 activity, presumably through reduced inhibitory activity of the lipocortins.

Confirmation of the relevance of lipocortins to the initiation of human parturition comes from the isolation from amniotic fluid of a protein with inhibitory activity against PLA_2 (T. Wilson *et al.*, 1985). A molecular weight of 80 kDa distinguishes it from the smaller lipocortins I and II, but in other respects the physical characteristics are similar. The protein is liberated during incubation of chorion, amnion and decidua, particularly from chorion (T. Wilson *et al.*, 1989). The activity of this protein, named gravidin, evident in medium from incubates of chorion taken before the start of labour, disappears in labour. Gravidin is a potent inhibitor of PLA_2, being active at concentrations as low as 10^{-10} M in dispersed whole-cell preparations of decidua as well as in cell-free preparations of porcine pancreatic PLA_2. It is not yet known whether gravidin is glucocorticoid dependent like lipocortin I and II, but the high concentrations of cortisol (10^{-5} M) required to inhibit the synthesis of $PGF_{2\alpha}$ in the endometrium (S.J.M. Skinner *et al.*, 1984) and the absence of effects of large doses of corticosteroids on the initiation of labour (Casey *et al.*, 1985) suggest that it is not. A possibility yet to be investigated is that gravidin is progesterone dependent, thereby providing an additional mechanism by which uterine quiescence and impeded PG synthesis is maintained by P_4 throughout pregnancy.

A P_4-dependent protein, blastokinin (Krishnan and Daniel, 1967) or uteroglobin (Beier, 1968), is a low molecular weight (15 kDa) molecule first found in the rabbit uterus in early pregnancy but is also present in other reproductive and non-reproductive tissues. It inhibits both chemotaxis of human neutrophils and thrombin-induced platelet aggregation. These effects depend, at least in part, on potent PLA_2 inhibitory activity, but the protein is genetically distinct from the lipocortins. A uteroglobin-like protein identified in late-luteal phase human endometrium and characterized both biochemically and immunologically (Kikukawa *et al.*, 1988) has yet to be studied in pregnancy. In the non-pregnant endometrium, the concentrations of uteroglobin and tissue PGE_2 and $PGF_{2\alpha}$ are inversely related and vary according to whether the endometrium is oestrogen or P_4 dominated. Another protein, interferon, has the potential for involvement in the control of prostanoid synthesis in pregnancy. Members of the interferon family, ovine trophoblastic protein 1 and bovine trophoblastic protein 1, are implicated in the inhibition of endometrial $PGF_{2\alpha}$ synthesis in early pregnancy in sheep and cattle that prevents luteolysis and permits implantation (sections 9.5 and 9.7). Recombinant 2α-interferon inhibits the production of PGE_2 and $PGF_{2\alpha}$ in explants of placenta and chorioamnion (G.E. Rice and S.P. Brennecke, personal communication) but has no effect on arachidonic acid-stimulated production, suggesting that the site of inhibitory action may be on a phospholipase.

9.2.9 SUMMARY – A MODEL

A soundly based model is not yet possible and the following proposal is necessarily speculative but may serve as a useful framework in which to incorporate future developments.

The earliest identifiable events in labour are increases in the production of $PGF_{2\alpha}$ and in the concentration of uterine oxytocin receptors; a relatively small increase in prostanoid production, perhaps PGE_2 by amnion, may stimulate oxytocin receptor formation, which leads in turn to a response to circulating oxytocin manifested in the myometrium by contractility and in the macrophage-like decidual stromal cells by PLC-mediated stimulation of $PGF_{2\alpha}$ production. The latter involves two pathways, one mediated by inositol triphosphate, which increases cytosolic Ca^{2+}, thereby activating PLA_2, and the other mediated by diacylglycerol, which activates protein kinase C, leading to activation of PGHS and to the phosphorylation and inactivation of gravidin, thereby enhancing further the activity of PLA_2. Positive-feedback loops involving prostanoid-enhanced oxytocin responsiveness and oxytocin-stimulated prostanoid synthesis in particular, lead to rapidly escalating release of $PGF_{2\alpha}$ from the abundant stores of arachidonic acid in the fetal membranes and decidua.

The signal to the foregoing events is unknown. Although, by analogy with experimental animals, the idea that the signal emanates from the fetus is appealing, no convincing evidence of fetal involvement, either by a circulating hormone or by a substance entering the amniotic fluid, has been forthcoming. The wide range but normal mean length of gestation in anencephalic pregnancies uncomplicated by polyhydramnios suggests that the fetus has, at most, a permissive role. Available evidence points strongly to the mechanism initiating labour being an intrauterine, paracrine system and consequently to the signal arising in the placenta/chorion. Local changes in oestrogen and P_4 metabolism could then alter the ratio of the two hormones to promote prostanoid release, but evidence that this occurs *in vivo* and of how it is regulated is lacking. Alternatively, the initiating system may be activated by a chorionic genetic clock transduced by protein kinases yet to be identified.

The release of $PGF_{2\alpha}$ from the decidua has multiple consequences for the uterus. The myometrium is stimulated not only by a direct action but also by potentiating the response to oxytocin and possibly by promoting the formation of gap junctions. The connective tissue of the cervix and uterine body undergoes complex biochemical changes that lead to softening and distensibility identical to the changes in response to relaxin; indeed, although circulating relaxin concentrations are unchanged at term, locally formed relaxin may mediate the action of prostanoids on connective tissues.

9.3 MONKEY

9.3.1 INTRODUCTION

Studies relevant to parturition have been made mainly of the rhesus monkey (*Macaca mulatta*) with a few of baboons (*Papio papio*), cynomolgus monkeys (*Macaca fascicularis*), the sooty mangabey (*Cercocebus atys*) and the common marmoset (*Callithrix jacchus*). The following account refers to rhesus monkeys unless otherwise specified.

The initiation of parturition in the monkey shares many features in common with human pregnancy, but human and non-human primates differ in the extent to which C_{18} and C_{19} steroids are 16α-hydroxylated in the fetus. The human fetus has very active 16α-hydroxylation, and consequently the major oestrogen entering the maternal circulation is oestriol with relatively small amounts of oestrone and oestradiol-17β, whereas non-human primate fetuses have low 16α-hydroxylase activity (Heinricks and Colas, 1970) and the major oestrogens are oestrone and oestradiol-17β. The physiological significance of this difference remains unexplained. Another consequence of the lack of placental 17α-hydroxylase (Ainsworth *et al.*, 1969) is the absence in primates of either a sharp fall

in P_4 production or rise in oestrogen production as the rate of conversion of P_4 to oestrogen is unresponsive to fetal cortisol concentrations.

9.3.2 ROLE OF THE FETUS

Maternal hypophysectomy is associated with normal parturition at term (Smith, 1954; Chez *et al.*, 1970), lending support to the generally held view that control of parturition in the monkey is intrauterine rather than a maternal endocrine function. As in human pregnancy, however, available evidence points to the fetus as having no more than a permissive or modulating role, implying that the controlling mechanism lies in the placenta and/or fetal membranes, although there is much less direct evidence to support this supposition than there is for human pregnancy.

Fetal hypophysectomy, performed by implanting radioactive capsules in the pituitary fossa (Chez *et al.*, 1970), is followed by labour at term. Surgical removal of the fetal head does not alter the mean duration of pregnancy (163 days *vs.* 167 days in controls) but alters the distribution, with births spread fairly evenly over a period of 50 days from 137 days onwards (Novy *et al.*, 1977). This pattern of deliveries is similar to that in human pregnancies complicated by anencephaly (W.M. Honnebier and Swaab, 1973). Fetal adrenalectomy has not been performed successfully, but continuous administration of glucocorticoids to the mother for 2–3 weeks before term in doses that inhibit fetal adrenal function as evidenced by low oestrogen production and adrenal hypoplasia is associated with normal-term delivery (Challis *et al.*, 1974a). More prolonged treatment with dexamethasone (for 5 weeks before term) leads to prolonged pregnancy in most, but not all, animals (Novy and Walsh, 1983; Mitchell *et al.*, 1984), which is consistent first with the non-involvement of fetal cortisol in the initiation of parturition and, second, with oestrogen having a permissive role.

Fetectomy is associated with placental retention to approximately term. Van Wagenen and Newton (1943) claimed that fetectomy did not alter the normal timing of placental delivery, but Lanman *et al.* (1975) found that fetectomy resulted in placental expulsion around term with a distribution similar to that occurring after fetal decapitation. Five baboons in which fetectomy was performed at 100–109 days delivered the placentas on day 168/9 (term 184 days) (Albrecht *et al.*, 1980). By contrast, Nathanielsz *et al.* (1990) reported that five monkeys fetectomized at 120–130 days' gestation retained the placenta for 21–32 days beyond term except for one delivered by hysterotomy at term because of cord prolapse. The results of the fetectomy and hypophysectomy experiments are consistent with a signal initiating parturition arising in the placenta and/or fetal membranes and with refinement of the precision of the signal being provided by the fetus, perhaps via the fetal zone of the adrenal.

9.3.3 PROGESTERONE

Progesterone (P_4) is secreted throughout pregnancy by both the placenta and the CL. Indeed, the concentration of P_4 in the uteroovarian vein is at least as high as that in the uterine vein (Walsh *et al.*, 1977a), but this does not reflect the relative production rates because of the much higher blood flow in the uterine veins. The CL continues to secrete P_4 after removal of both the fetus and placenta by Caesarean section in hypophysectomized monkeys, although at a much reduced rate (Walsh *et al.*, 1977b). Since fetectomy alone does not cause a fall in uterine vein or peripheral P_4 concentrations, it is likely that CL function throughout pregnancy is normally maintained by a product of the placenta, probably chorionic gonadotrophin. Luteolysis preceding parturition is unlikely in view of the persistence of P_4 secretion from the CL after Caesarean section.

The mean concentration of P_4 in maternal

plasma shows little change throughout most of pregnancy (Figure 9.2).

Immediately prepartum, the concentration either remains unchanged (Stanczyk *et al.*, 1986) or rises inconsistently (Challis *et al.*, 1974b). P_4 concentrations follow a similar pattern in baboons (Albrecht, 1980; Albrecht *et al.*, 1980), cynomolgus monkeys (Stabenfeldt and Hendrickx, 1973a; Hodgen *et al.*, 1977), bonnet monkeys (*Macaca radiata*) (Stabenfeldt and Hendrickx, 1972) and the sooty mangabey (Stabenfeldt and Hendrickx, 1973b). In none of these species is there evidence of a fall in plasma P_4 concentrations before parturition. There is a different pattern of concentrations of P_4 and oestradiol-17β in common marmosets (Chambers and Hearn, 1979; and Torii *et al.*, 1989; Figure 9.3).

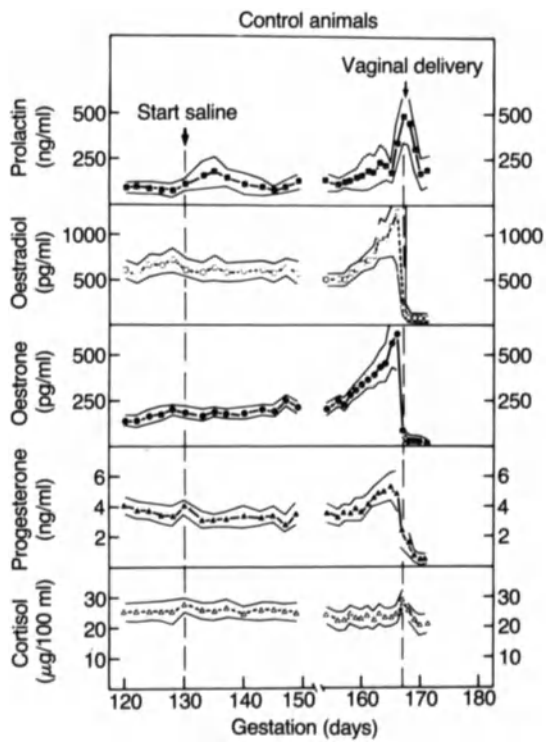

Figure 9.2 Maternal venous concentrations of hormones in rhesus monkeys from Day 120 of gestation until spontaneous delivery at term. (Reproduced with permission from Novy and Walsh, 1983.)

P_4 remains at levels similar to the luteal phase until about 90 days of pregnancy, when values increase rapidly to a peak at 110–130 days (term 144 days). Concentrations then fall steeply to values approximating those of early pregnancy 4–10 days before term and to undetectable levels at parturition. The marmoset is the only primate in which a marked prepartum fall in plasma P_4 concentrations has been reported. The marmoset placenta secretes P_4 from early pregnancy (Hodges *et al.*, 1983). The high levels in late pregnancy are considered by Torii *et al.* (1989) to be of placental origin, and the prepartum fall probably results from reduced synthesis of P_4.

Both the mother and the fetal monkey show marked diurnal variations (up to 100%) in plasma P_4 concentrations with a peak at 22:00–24:00 h and a trough at 08:00–10:00 h (Walsh *et al.*, 1984), which is the reciprocal of the diurnal rhythm of cortisol concentrations in the maternal plasma (Figure 9.4).

The changes in P_4 concentration are thought to be due in part to alterations in the rate of secretion of pregnenolone by the fetal adrenal and in part to displacement of P_4 from cortisol-binding globulin by high concentrations of cortisol in the morning. A similar diurnal variation in the fetal levels of DHEAS coincides with that of P_4, and both are thought to be a passive response of the fetal adrenal to inhibition of fetal ACTH secretion by the maternal diurnal rhythm of plasma cortisol, which readily crosses the monkey placenta (Novy and Haluska, 1988). Uterine activity also exhibits a diurnal rhythm during the week before term with an increase during the hours of raised P_4 and DHEAS concentrations from 22:00–02:00 h. However, the association is not causal since the fluctuations in P_4 and DHEAS are abolished by infusing the fetus with ACTH, but the diurnal rhythm of uterine activity persists although it is abolished by maternal adminis-

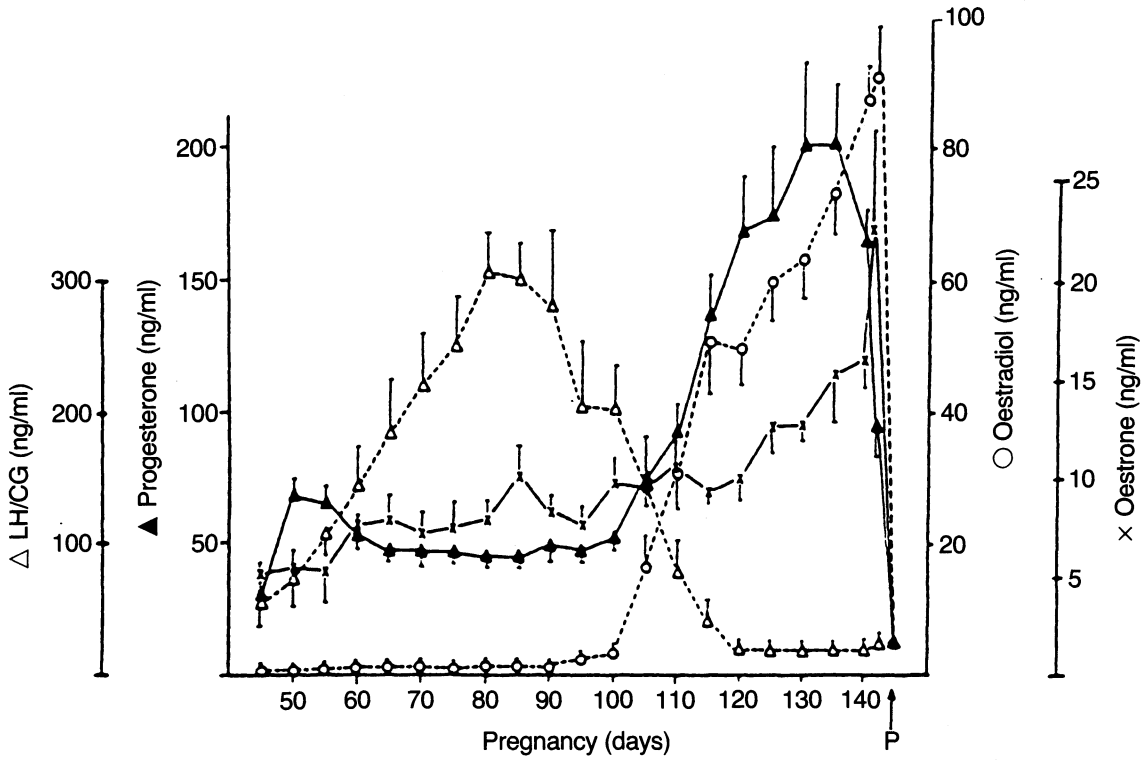

Figure 9.3 Maternal peripheral plasma concentrations of progesterone (▲), oestradiol 17β (○), oestrone (×) and LH/CG (△) hormones during pregnancy in marmoset monkeys. P= parturition. (Reproduced with permission from Chambers and Hearn, 1979.)

tration of dexamethasone (Ducsay *et al.*, 1983; Walsh *et al.*, 1984).

Haluska *et al.* (1987a) found that oral administration of mifepristone (20 mg/kg/day) for three doses at about 130 days of gestation was followed by a sustained increase in uterine activity until Caesarean section was performed after 48 h. Uterine activity (predetermined by intra-amniotic pressure and EMG) differed from normal labour in that the contractions were of longer duration (38 min) and lower frequency (5/h). The concentration of $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGFM and bicyclo-PGE_2 in amniotic fluid increased, but not until 40 h after the onset of uterine activity. The cervix did not soften or dilate. These results differ from those of Wolf *et al.* (1989), who gave a single oral dose of mifepristone (25 mg) to 19 cynomolgus monkeys on day 160 of pregnancy (term 167 days): they observed slight (0.5–1.0 cm) cervical dilatation in 15 animals, but uterine activity (by manual palpation) was noted in only two animals, both of which delivered within 48 h. The reason for these disparate results is uncertain but is unlikely to be attributable to the difference in species or to differing gestational ages. It is clear, however, that withdrawal of P_4 action in late pregnancy fails to induce normal parturition in either species although P_4 receptors are blocked and oestradiol receptors increase after treatment with mifepristone (Haluska *et al.*, 1990a).

9.3.4 OESTROGEN

The fetal monkey has a functional fetoplacental unit in which oestrogen synthesis by the placenta depends on a supply of androgens (androstenedione and DHEAS) by the fetal adrenal (Walsh *et al.*, 1980). Inhibition of adrenal activity by maternal dexamethasone administration decreases oestrogen and androgen concentrations in the mother and fetus. Conversely, stimulation of the fetal adrenal with exogenous ACTH while endogenous ACTH is inhibited by dexamethasone sharply increases androgen and oestrogen levels. Oestrone is the major oestrogen in the fetal circulation and oestradiol-17β predominates in the mother (Challis *et al.*, 1974b; Resko *et al.*, 1975). The concentrations of oestrone and oestradiol-17β remain constant during the last third of pregnancy until the last few days, when the concentration of both oestrogens rise by approximately 10% (Walsh *et al.*, 1979) (Figure 9.2). Similar patterns of oestrone and oestradiol-17β in the maternal circulation are present in the baboon (Albrecht *et al.*, 1980) and cynomolgus monkey (Hodgen *et al.*, 1977). Like P_4, plasma concentrations of oestradiol-17β in marmosets differ from those of other monkeys in that values do not rise immediately prepartum (Torii *et al.*, 1989): There are very low

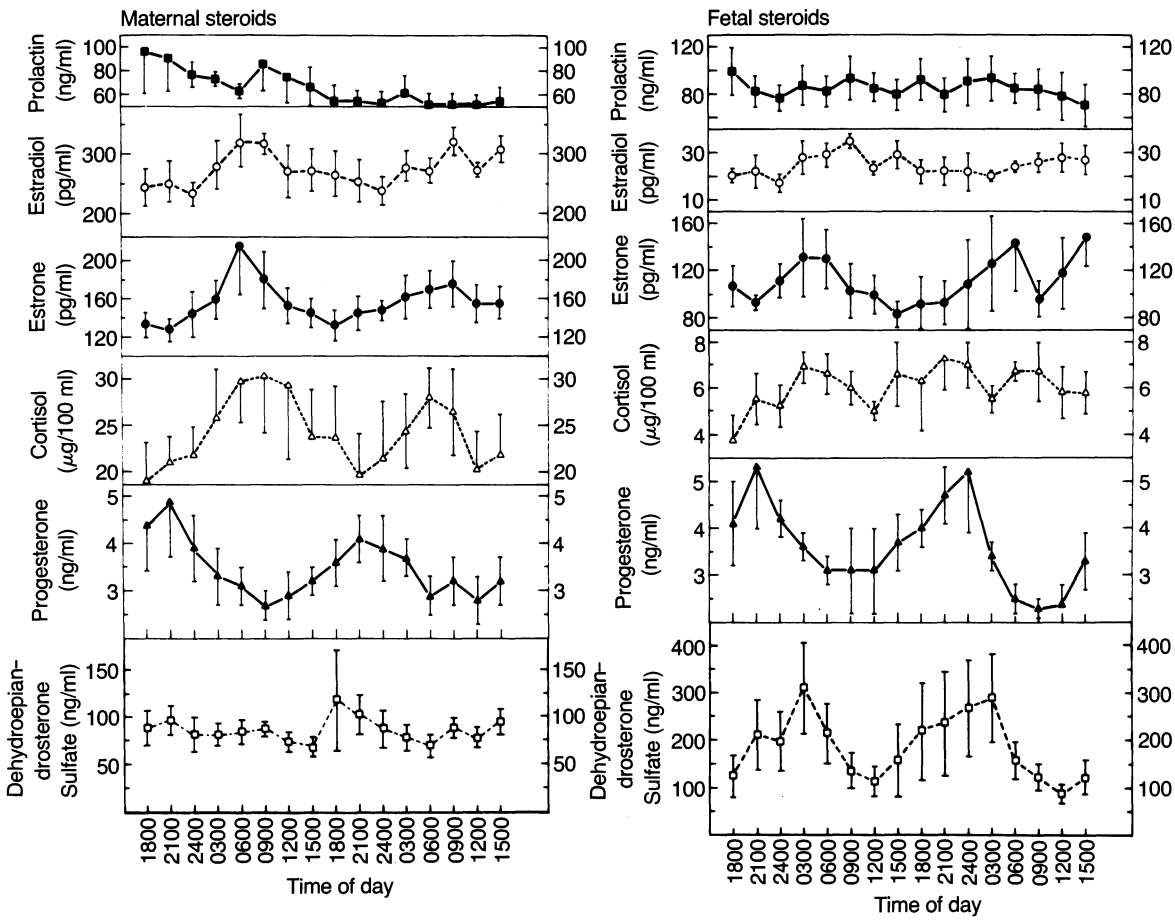

Figure 9.4 Hormone fluctuations in maternal and fetal peripheral plasma every 3 h during a 48 h period in long-term catheterized rhesus monkeys. (Reproduced with permission from Walsh *et al.*, 1984.)

concentrations before 90 days, peak values at 110–130 days and a trend to falling levels in some animals at term (Figure 9.3). Walsh *et al.* (1980) proposed that the terminal rise in oestrogen concentrations in rhesus monkeys provides a mechanism for a fetal effect on the timing of parturition. Experimental anencephaly reduces oestrogen concentrations and disturbs the distribution of pregnancy lengths (Novy *et al.*, 1977) and the administration of oestrogen induces delivery in monkeys with prolonged pregnancies and low oestrogen levels as a result of fetal death. Subsequent experiments by these and other workers make it unlikely that the preparturient rise in oestrogen triggers parturition, although it probably reflects an increase in adrenal activity and cortisol secretion that has an important role in promoting the final maturation of the fetal lungs and other organs. Inhibition of the rise in oestrogen by maternal dexamethasone treatment does not alter pregnancy length (Challis *et al.*, 1974a). Raising circulating oestrogen concentrations to very high levels by maternal treatment with oestradiol fails to initiate labour (Novy and Walsh, 1983). Insertion of Silastic capsules loaded with oestradiol into the amniotic sac is also ineffective (Novy and Haluska, 1988). When fetal monkeys were infused with ACTH from 125–135 days of gestation until delivery, there were several-fold increases in the concentrations of cortisol, DHEAS and oestradiol-17β, but delivery occurred normally at term (Novy and Haluska, 1988). In baboons, prolonged administration of the anti-oestrogen, ethamoxytriphetol, does not alter pregnancy length (Albrecht, 1980). However, the concomitant increase in maternal plasma concentrations of oestradiol-17β and fall in P_4 suggest that (1) oestrogen synthesis in the baboon placenta is subject to feedback regulation and (2) oestrogen is required for sustained high production of P_4.

9.3.5 OXYTOCIN

The concentrations of oxytocin are low in maternal plasma and amniotic fluid throughout the second half of pregnancy in rhesus monkeys and show no upward trend towards term (M.D. Mitchell *et al.*, 1980b).

A diurnal rhythm of oxytocin in maternal plasma coincides with that of P_4 and of uterine activity (M.B.O.M. Honnebier *et al.*, 1989a). The nocturnal uterine activity is abolished by a single bolus of a competitive oxytocin antagonist, suggesting a causal relationship between oxytocin concentrations and uterine activity at 98–117 days' gestation. Moreover, sensitivity to exogenous oxytocin is greatest at the time of nocturnal uterine activity (M.B.O.M. Honnebier *et al.*, 1989b).

In baboons, oxytocin concentrations in maternal peripheral plasma are similar to those in rhesus monkeys but are more variable and show a weak positive correlation with gestational age (Dawood *et al.*, 1979b). The concentration in uterine vein plasma is significantly higher than that in peripheral plasma, and the values in umbilical vein blood and fetal jugular blood sampled at the time of Caesarean section (three animals near term) are substantially higher than in maternal blood. Maternal infusion of oxytocin at a rate of 1–40 miu/min near term causes a dose-related increase in uterine activity beginning with the smallest dose. A bolus dose of 10 miu of oxytocin injected into the umbilical vein at the time of Caesarean section is followed within 15 min by a transient increase in concentration in maternal plasma and a rise in concentration in amniotic fluid. A massive intravenous bolus of oxytocin (20 U) given to cynomolgus monkeys near term failed to induce labour (Wolf *et al.*, 1989).

Hirst *et al.* (1991) did not confirm fetomaternal passage of oxytocin even when infused into the fetus in large amounts. The concentration of oxytocin in maternal plasma increased from 136 days, but only in samples

taken at night, and a further increment was noted in labour. Oxytocin receptors increased fourfold from 130 days to the early postpartum period. Hirst *et al.* (1991) concluded that a combination of increased concentrations of plasma oxytocin and myometrial oxytocin receptors facilitates delivery.

9.3.6 RELAXIN

In the rhesus monkey (Weiss *et al.*, 1981) and the baboon (Castracane *et al.*, 1985), relaxin becomes detectable in the circulation early in pregnancy and persists at constant levels to term. Although the source of circulating relaxin in these species is the CL (Ottobre *et al.*, 1984), relaxin remains detectable in decidua, placenta and myometrium after luteectomy (Castracane *et al.*, 1985), suggesting that local production may contribute to the physiological role of relaxin.

9.3.7 EICOSANOIDS

A role for PGs in the initiation of parturition in monkeys is supported by evidence similar to that in human pregnancy. Administration of PGE_2 or $PGF_{2\alpha}$ induces premature labour or abortion in rhesus monkeys (Kirton *et al.*, 1970; Challis *et al.*, 1974b), although extra-amniotic injection of arachidonic acid fails to do so (J.S. Robinson *et al.*, 1978). Daily intramuscular injections of $PGF_{2\alpha}$ at 142–151 days of gestation is followed by delivery in 2–6 days. Conversely, treatment with the PG synthase inhibitor, indomethacin, prolongs pregnancy (Novy *et al.*, 1974). However, few studies that systematically investigated eicosanoids in monkeys at term and in labour have been reported and information is fragmented and incomplete. The most detailed report is that of M.D. Mitchell *et al.* (1976), who measured PGF and PGFM in peripheral plasma and amniotic fluid of rhesus monkeys in late pregnancy. The concentration of PGFM in plasma is similar to that in non-pregnant animals (Cornette *et al.*, 1974) and falls significantly in the last 5 days of pregnancy. At least up to 24 h before parturition no increase in circulating PGFM is present. In amniotic fluid, however, both PGF and PGFM increase fourfold in the last 5 days of pregnancy (M.D. Mitchell *et al.*, 1976; Dubin *et al.*, 1988). When pregnant monkeys were treated with betamethasone for 2 weeks from 120 days, the low concentration of amniotic fluid PGFM at 133 days in controls was unaffected by treatment, but at 160 days, when PGFM had increased substantially in controls, the concentrations in betamethasone-treated animals remained low (Dubin *et al.*, 1988). Oestrone, oestradiol-17β and P_4 concentrations were unaffected by treatment, whereas cortisol levels were markedly reduced. Cortisol concentrations had returned to control values at 160 days. Maternal plasma levels of oestradiol-17β were suppressed during treatment but did not differ from controls at 160 days. The authors speculate that the low amniotic fluid concentrations of PGFM 4 weeks after discontinuing treatment with betamethasone may be due to induction of a protein that inhibits PLA_2 or PG synthase. The fetal adrenals failed to recover from suppressed growth.

Walsh (1991) found that $PGF_{2\alpha}$ in amniotic fluid was undetectable until 1–2 weeks before delivery and increased in association with a nocturnal pattern of strong uterine activity in two of five animals; two other animals began labour and delivered with very little increase in $PGF_{2\alpha}$ levels. In contrast, the concentrations of 5-HETE and LTC_4 were higher and rose markedly and consistently up to 7 days before the start of labour. There is a rise in amniotic fluid levels of 5-HETE and a similar increase in LTB_4 but no change in 15-HETE; similar but not significant changes in these lipoxygenase metabolites were observed after the maternal administration of mifepristone. Walsh (1991) suggests that 5-HETE and LTC_4 may stimulate uterine contractions or have chemotactic effects. LTC_4 may lack oxytocic effects in monkeys since it is ineffective in

stimulating human myometrial strips *in vivo* (Bryman *et al.*, 1985; Lopez-Bernal *et al.*, 1989), but 5-HETE stimulates human myometrial tissue in a dose-dependent manner (Bennett *et al.*, 1987) and could contribute to the initiation of labour in monkeys.

Amnion, chorion, decidua and myometrium obtained by Caesarean section at 140–168 days of pregnancy all release PGs in a superfusion system (M.D. Mitchell *et al.*, 1978d). The major product at all gestational ages is PGE from amnion. Decidua parietalis releases increased quantities of PGF and PGFM towards term, their total approximating half that of PGE from amnion at 168 days. In all tissues, the ratios of PGE/PGF and PGF/PGFM increase at term.

The role of eicosanoids in determining the types of uterine activity immediately preceding the onset of parturition remains unclear. Uterine contractility in pregnant monkeys is of two types:

1. Periods of EMG activity lasting for at least 3 min at least throughout the second half of pregnancy and showing no diurnal rhythm; and
2. contractions of <1 min in duration make their appearance in late pregnancy.

The latter have a strong diurnal rhythm with peak activity between 22:00 and 02:00 h (Ducsay *et al.*, 1983), which coincides with the peaks in the concentrations of P_4 and DHEAS in the fetal circulation. At approximately 1 week before parturition the nocturnal periods of uterine activity increase progressively to a crescendo 48–72 h before delivery. Novy and Haluska (1988) suggest that parturition in this species is an extension of a normally occurring nocturnal pattern of uterine activity in late pregnancy. The diurnal rhythm of uterine activity persists in animals in which the diurnal pattern of secretion of DHEAS is abolished by fetal infusion of ACTH, thus excluding the fetal adrenal as the source of the rhythm. In monkeys treated with RU486, an exaggerated diurnal rhythm of uterine activity was observed during the first 24 h but thereafter activity increased steadily without evidence of diurnal variation (Haluska *et al.*, 1987a). The cause of the diurnal rhythm is unknown, although it probably has important implications for understanding the mechanism initiating parturition in non-human primates.

9.3.8 SUMMARY

Many aspects of parturitional physiology in monkeys have features in common with human pregnancy, and it seems likely that a paracrine mechanism is involved in both species. The monkey fetus seems to have little more than a permissive role, as evidenced by a lack of consistent effects on pregnancy of length of hypophysectomy, decapitation, corticosteroid treatment or stimulation of the fetal adrenals with ACTH. Oestrogen and P_4 show no pronounced changes prepartum, and experimental manipulation of their concentration has no consistent effects in initiating or delaying parturition. The postulated paracrine system is further supported by the tendency of the placenta to remain *in situ* after removal of the fetus until approximate term or later in monkeys and 2 weeks before term in baboons. Overall, however, ignorance of the mechanism initiating parturition in non-human primates is even greater than that in humans.

9.4 PIG

9.4.1 INTRODUCTION

The commercial importance of the pig industry in most countries has ensured that considerable research funds have been directed into pig research. The relatively high perinatal mortality in this species has led to an emphasis on parturitional research. Not only is the pig a CL-dependent species, but it is also polytocous and so, if more than one of

the fetuses initiates parturition, how are their actions coordinated?

In ruminants, activation of the hypothalamic–pituitary–adrenal axis increases fetal plasma cortisol concentrations, initiating parturition and causing the synchronous maturation of the key organs essential for post-natal survival. In pigs, the two processes may be separate but, if so, they must be linked in some way to ensure that the piglets are mature at the time of birth. The pig represents a great technical challenge for fetal physiologists; not only are the fetuses small (smaller than fetal lambs) but there are so many. Moreover, relaxin is found in pigs; it is easily measured, is released from the CL and inhibits uterine activity.

9.4.2 PROGESTERONE

(a) Corpus luteum

The maintenance of gestation in the sow depends on the continued secretion of P_4 by the ovary. The placentas may make a small contribution to P_4 production during late pregnancy but ovariectomy performed at any time during gestation rapidly terminates pregnancy, indicating that placental P_4 production is inadequate for the maintenance of pregnancy (du Mesnil du Buisson and Dauzier, 1957). Progesterone replacement therapy will prevent abortion after ovariectomy (Fèvre *et al.*, 1968; First and Staigmiller, 1973), and the induction of additional CL on day 103 or 107 prolongs gestation (Bosc *et al.*, 1974; Martin *et al.*, 1977). Between four and six CL are needed for the production of sufficient P_4 to maintain late pregnancy (Martin *et al.*, 1977). Abortion occurs if maternal P_4 falls below 4 ng/ml (Ellicott and Dziuk, 1973; Nara *et al.*, 1981).

The length of gestation in the sow is 112–116 days. Maternal plasma P_4 concentrations fall slowly over the last 10–14 days of gestation before decreasing rapidly in the final 24–36 h before farrowing (Robertson and King, 1974; Baldwin and Stabenfeldt, 1975; Silver *et al.*, 1979a).

(b) Placental synthesis

There is clear evidence that the placenta produces P_4 during late pregnancy. Fetal P_4 concentrations are higher than maternal concentrations (Barnes *et al.*, 1974; Silver *et al.*, 1979a; Hagen *et al.*, 1983). The maintenance of high fetal P_4 concentrations in fetuses in ovariectomized sows (Randall *et al.*, 1986) and in sows after luteolysis induced with cloprostenol (Silver *et al.*, 1983) indicates that uterine uptake and placental transfer of P_4 from the mother are unlikely. There is a negative arteriovenous difference for P_4 across the umbilical vessels, suggesting placental production of P_4 (Hagen *et al.*, 1983; Nase *et al.*, 1985), and the fetal placenta can synthesize P_4 from P_5 *in vitro* (Craig, 1982). This confirmed histochemical (Christie, 1968; Dufour and Raeside, 1969) and biochemical (Ainsworth and Ryan, 1966) evidence of 3β-HSD in the pig placenta. The source of P_5 is not known: cholesterol may be synthesized *in situ* or taken up from the maternal circulation and then converted to P_5, or P_5 may be derived from the maternal or fetal circulations. There is no information on the circulating concentrations of P_5 in the sow.

When the luteal source of P_4 is removed (by ovariectomy) and replaced by medroxyprogesterone acetate (MPA) (Craig, 1982), there is a negative arteriovenous difference for P_4 across the maternal placental vessels. Maternal P_4 concentrations increased from day 104 to 110 and then decreased until the expected day of delivery (day 114). There are potential problems with the use of MPA because it depresses ovarian production of P_4 during the luteal phase in human (Johansson, 1971; Shinada *et al.*, 1978) and rats (Sunde *et al.*, 1982). However, the administration of MPA to sows in late pregnancy did not suppress P_4 production as judged by maternal P_4 concentrations (Whitely *et al.*, 1990),

and there is a negative arteriovenous difference for P_4 in the umbilical vessels of fetuses of ovariectomized sows (Randall *et al.*, 1986).

The major source of P_4 in the fetal circulation therefore appears to be the fetal placenta. The contribution of the fetal adrenal to the fetal P_4 pool would seem to be minimal (MacDonald *et al.*, 1980). However, in the last 24 h of gestation, when there is a sharp fall in maternal P_4 levels, fetal P_4 concentrations rose in both arterial and umbilical venous blood, presumably due to increased synthesis not decreased metabolism of P_4. Flint *et al.* (1979) noted a decrease in the formation of polar metabolites (pregnanetriols and pregnanediols) from P_4 by the term placenta, but the presence of high P_4 concentrations in samples taken from piglets at birth suggests that the P_4 may be derived from the adrenal which is maximally stimulated at this time (Silver *et al.*, 1979b). High concentrations of P_4 are also seen in neonatal pigs after premature delivery induced with cloprostenol (Silver *et al.*, 1983), suggesting that in piglets (as in foals) cortisol induction of 17α-hydroxylase activity in the fetus occurs late in gestation, but is not complete in premature newborn (Thorburn, 1993).

(c) Placental metabolism

Despite the ability of the placenta to produce P_4 during late pregnancy, it does not significantly contribute to maternal P_4 concentrations and cannot maintain the pregnancy in the absence of the CL. Indeed, there is a marked positive arteriovenous difference across the maternal placental vessels (6–8 ng/ml) which indicates that the uterus clears a large amount of P_4 from the maternal circulation. The fate of this P_4 is unknown, but it is presumably metabolized by the placenta, endometrium and myometrium. The major products of P_4 metabolism by the endometrium (maternal tissue) *in vitro* were 5β-reduced pregnanediols (3α,20α-diol) and pregnanetriols (3α,17α,20α-triol) together with 5β-pregnan-3α-ol-20-one (5β-pregnanolone). Less than 10% of the metabolites were 5α-reduced (Craig, 1982). In contrast, the products of placental (maternal or fetal tissues) metabolism were predominantly 5α- and 5β-pregnanolones (3α-hydroxy-5-pregnan-20-one). 17α-Hydroxylated products accounted for <5% of the metabolites isolated. Since the placenta produces mainly 5α- and 5β-pregnanolones, it presumably lacks the 20α-reductase enzyme. This study suggests that in the maternal endometrium the initial reduction of P_4 is to the 5β derivatives by an NADPH-requiring enzyme and the subsequent reduction of the 3-ketone is predominantly to the 3α-hydroxysteroid (5β-pregnenolone). A further reduction of the 20-ketone function to a 20α group leads to the formation of pregnanediol. The endometrium can also convert the 17α-hydroxylated derivative of P_4 through the above steps to pregnanetriol (5β-pregnane-3α,17α,20α-triol).

Whether these pathways operate *in vivo* to metabolize the large amounts of P_4 secreted by the CL into is not known. It is assumed that P_5 (and P_4) are metabolized via the Δ_4 pathway (Craig, 1982), although the Δ_5 pathway is primarily operative in the sheep (Nathanielsz *et al.*, 1982; Mason *et al.*, 1989), suggesting that the Δ_5 is the major pathway. However, the Δ_4 pathway may be functional close to term because the placenta shows increased ability to metabolize P_4 to 17α-OH-P_4 at this time.

The magnitude of the placental production of P_4 and its metabolites is still not clear. In the goat, Sheldrick *et al.* (1981) have proposed that the placental production of P_4 increases progressively from day 60 of gestation and reaches high levels but, in contrast to the sheep, most of this P_4 is metabolized to metabolites such as 5β-pregnanediol. Based on the plasma concentrations of unconjugated oestrone and oestradiol-17β in the pregnant sow, placental steroid biosynthesis commences about Day 80 of gestation. Craig

(1982) implied that increasing amounts of 5β-reduced metabolites are secreted by the pig placenta, but this has not been confirmed. Steroidogenic activity in the pig placenta may be low, with most of the P_4 synthesized by the placenta being directly converted to oestrogens. The placenta (and the endometrium) may play a much greater role in the clearance and metabolism of CL-derived P_4. There is a need to undertake with pigs the experiments undertaken with goats, whereby pregnancy is maintained after ovariectomy with MPA and P_4 and its major metabolites measured by specific radioimmunoassays.

9.4.3 LUTEOTROPHINS

(a) Protein hormones

Pituitary stalk section of hysterectomized sows, or sows in early pregnancy, results in luteal regression and abortion (L.L. Anderson *et al.*, 1967), indicating that continuous secretion of pituitary luteotrophin is necessary to maintain the CL and that the possible secretion of a placental luteotrophin is inadequate to replace the function of the pituitary. In hypohysectomized/hysterectomized sows, the CL can be maintained by LH alone and LH, but not prolactin, can prevent luteal regression (L.L. Anderson *et al.*, 1966). Administration of antiserum to LH causes luteal regression (Spies *et al.*, 1967). LH is therefore important for CL maintenance during the first half of pregnancy. However, prolactin may play a role in CL maintenance during mid- to late pregnancy: pituitary stalk section after day 70 of gestation or hypophysectomy resulted in regression after about 20 days (du Mesnil du Buisson, 1966), and hypophysectomy in late pregnancy terminated the pregnancy (Kraeling and Davis, 1974). Injections of bovine prolactin, but not LH, maintain luteal function (weight and P_4 content) after hypophysectomy of sows between days 70 and 80 (du Mesnil du Buisson and Denamur, 1969).

The administration of bromocriptine, a dopamine agonist, to late pregnant (day 111) sows reduced prolactin concentrations to <2 ng/ml, and caused the early onset of luteolysis in two out of three animals, again suggesting a luteotrophic role for prolactin in late pregnancy (Taverne *et al.*, 1982). There is also an increasing density of prolactin binding sites in luteal cells during the first half of pregnancy in the sow (Rolland *et al.*, 1976). It is not known whether antiserum to prolactin antiserum after day 70 would cause abortion.

The sudden decrease in P_4 secretion just before parturition may be related to the removal of a pituitary luteotrophic stimulus. The most likely pituitary luteotrophin in late pregnancy is prolactin, but there is no evidence of a preparturient decrease in plasma prolactin concentrations. Indeed, an increase in the maternal prolactin concentration has been reported (van Landeghem and van der Wiel, 1978; Taverne *et al.*, 1979a, 1982; Dusza and Krzymowska, 1981).

(b) Oestrogens

Administration of oestrogens during the oestrous cycle prolongs luteal function in pigs (Kidder *et al.*, 1955; Gardner *et al.*, 1963; Kraeling *et al.*, 1975) and increases plasma P_4 concentration (Ford *et al.*, 1982). One possible mechanism is that oestrogen reduces $PGF_{2\alpha}$ release into the circulation, thereby blocking the normal luteolytic action of the uterus (see Bazer *et al.*, 1986); such a mode of action has been suggested for the luteotrophic effect of endogenous oestrogen secreted by the conceptus early in gestation (Bazer and Thatcher, 1977). Oestrogen administered on day 12 after oestrus, apart from extending the lifespan of the CL in non-pregnant gilts, also increased the level of unoccupied LH receptors (Garverick *et al.*, 1982). Since maintenance of the CL is dependent on LH in the pig from about day 14 after oestrus (du Mesnil du Buisson, 1966, 1973; L.L. Anderson *et al.*, 1967; Spies *et al.*, 1967), the luteotrophic

action of oestrogen may be manifested by increasing numbers of LH receptors and thus LH action. This would be consistent with the lack of action of oestrogens in maintaining CL after hypophysectomy (du Mesnil du Buisson, 1966) or pituitary stalk section (L.L. Anderson *et al.*, 1967).

Conley and Ford (1989) demonstrated a direct luteotrophic effect of oestrogen on the pig CL *in vivo* by the intraluteal implantation of oestradiol. However, the dose of oestradiol used was much higher than that necessary to stimulate CL function when administered by an intrauterine route (Ford *et al.*, 1982). Conley and Ford (1989) concluded that the systemic effect of oestradiol noted in their study was due to an effect on the uterus by reducing $PGF_{2\alpha}$ release into the utero-ovarian vein as described by Frank *et al.* (1977) and Ford *et al.* (1982). This supports the proposal by Bazer *et al.* (1986) that the primary effect of oestrogen that results in maintenance of luteal function is a decrease of the luteolytic signal. Against a direct action of oestrogen on the CL was the finding that oestrogens did not increase the lifespan of CLs of pituitary stalk-sectioned sows (L.L. Anderson *et al.*, 1967), although it is possible that there were low LH values in these animals. Consistent with a direct action of oestrogen on the CLs was the stimulatory action of oestrogen on P_4 production by pig granulosa cells in culture (Goldenberg *et al.*, 1972; Veldhuis *et al.*, 1981) and of stilboestrol on binding of LH by isolated pig granulosa cells (Nakano *et al.*, 1977).

Garverick *et al.* (1982) suggested the following sequence of events. Firstly, before day 12 of gestation, the small amounts of oestrogen secreted by the conceptus act directly to inhibit endometrial $PGF_{2\alpha}$ synthesis and thus reduce the luteolytic signal. Secondly, the increasing concentrations of oestrone sulphate in maternal plasma during early pregnancy (Robertson and King 1974; Heap *et al.*, 1981) would explain the rise in luteal LH receptors between days 20 and 30 of gestation (Ziecik *et al.*, 1980).

It is not known whether oestrogens are luteotrophic after day 70 of gestation in the pig. Administration of oestradiol-17β to two pregnant gilts (days 102 and 105 of gestation) (Flint *et al.*, 1979) had no luteolytic or luteotrophic effects, but it is not clear how the luteotrophic effect was assessed because hormone concentrations are not indicated.

Oestrogen concentrations increase in the maternal circulation from day 70 to term (Robertson and King, 1974) and may act with prolactin to maintain luteal function. There appears to be neither a placental lactogen nor a chorionic gonadotrophin in the pig in early or late pregnancy (P.A. Kelly *et al.*, 1976; Flint *et al.*, 1979; A.P.F. Flint and P. Saunders, unpublished observations).

Considerable evidence is available indicating that fetuses may play a part in maintaining the CL of pregnancy to term (summarized by First and Bosc, 1979). At least four fetuses are required to maintain a pregnancy (Polge *et al.*, 1966), but when one uterine horn is removed, two fetuses are sufficient (Dhindsa and Dziuk, 1968). Pregnancy can be maintained for only one fetus when all the uterus except that portion occupied by the fetus is removed by day 14 of pregnancy (du Mesnil du Buisson and Rombauts, 1963). When all fetuses were killed after day 30, CL and P_4 concentrations were maintained until day 60 (Webel *et al.*, 1975) or from days 100 to 120 of gestation (Coggins and First, 1977) if the fetuses had not been resorbed. However, removal of all fetuses at day 102 caused delivery of the placentas in less than 48 h (Chiboka *et al.*, 1976). First and Bosc (1979) concluded that fetal tissue or a product of the intact conceptus, such as oestrogen (Bazer and Thatcher, 1977), must be present *in utero* to prevent the uterus from initiating luteolysis. Embryo extracts are luteotrophic when infused into the uterine lumen of unilaterally pregnant sows: luteal function is maintained and $PGF_{2\alpha}$-induced luteal regression is retarded (Longenecker and Day, 1972; Ball and day, 1982a). Ball and Day (1982b) used intra-

luteal implants containing pig embryonic extracts to maintain the function of an individual CL in an ovary, suggesting that the embryo extract can (also) directly prevent the luteolytic effects of the uterus at the level of the CL. The active principle(s) was heat stable and charcoal extractable, indicating that it was of low molecular weight, possibly a steroid (e.g. oestradiol). However, PGE_2 is another luteotrophic factor produced by the pig embryo (Akinlosotu *et al.*, 1986) as in sheep (Henderson *et al.*, 1977).

(c) Prostaglandin E_2

PGE_2 has been proposed as one of the signals for maternal recognition of pregnancy in sheep and cows (sections 9.5 and 9.7). In contrast, when intrauterine infusions of PGE_2 are started on days 12–14 of the oestrous cycle in cyclic pigs, luteal function is not prolonged (Schneider *et al.*, 1983), but if the infusion is started earlier in the cycle (day 7) and continued through to day 23 the decrease in plasma P_4 concentrations that normally occurs around day 15 is delayed and luteal function extended (Akinlosotu *et al.*, 1986). In the PGE_2-infused gilts, peak P_4 concentrations remained high from day 9 until day 23, and rapidly declined to <1 ng/ml by day 26.

The exact mechanisms of action of PGE_2 in maintaining luteal function are still unknown. Like LH, PGE_2 stimulates P_4 secretion and cyclic AMP production by bovine luteal cells *in vitro* (Speroff and Ramwell, 1970; Marsh, 1971) but the PGE_2 analogue, sulproston, is neither luteotrophic nor luteolytic in the cyclic pig (Sander *et al.*, 1982). This analogue is believed to act via the EP_1-type PGE_2 receptor and stimulate IP_3 turnover (R.A. Coleman *et al.*, 1985) and not via the EP_2 receptor, which activates adenylate cyclase and increases cAMP. In cycling ewes, PGE_2, by arterial infusion, induces luteolysis (section 9.5).

The luteotrophic effect of the intrauterine infusion of PGE_2 (Akinlosotu *et al.*, 1986) may be mediated by an action on the endometrium, inhibiting the release of the luteolysin $PGF_{2\alpha}$ into the uterine venous drainage, or by a direct luteotrophic action on the CL of pregnancy. The putative luteotrophic action of PGE_2 on the CL would require that, for the latter, adequate levels of PGE_2 would need to reach the CL, but information is lacking on the plasma concentrations of PGE_2 in the early pregnant pig.

Does PGE_2 continue to exert a luteotrophic role throughout pregnancy and does it act locally on the endometrium to inhibit $PGF_{2\alpha}$ release? The placenta is an important source of PGE during late gestation in the pig. PGE_2 is secreted into the fetal circulation [mean PGE concentration in the umbilical vein at birth being 11.3 ± 3.3 ng/ml (34 nmol/l) compared with 3.0 ± 0.3 ng/ml in the arterial plasma], and in catheterized fetuses from late pregnant sows PGE_2 levels are high in the umbilical vein (Silver *et al.*, 1979a). Significant amounts of PGE_2 are present in the maternal uterine vein at farrowing (4.0 ng/ml, 12.0 nmol/l) (Silver *et al.*, 1979a). Considering the high placental blood flow at that time, the production rate of PGE_2 by the late pregnant uterus is very high. PGE_2 is also found in the maternal artery during late pregnancy (1.4 ± 0.3 ng/ml, 4.2 nmol/l), indicating that considerable amounts of PGE_2 had escaped the lungs and could be exerting a luteotrophic effect on the CL of pregnancy. PGE_2 may also exert a stimulatory action on the myometrium and may explain the increase in uterine activity during the last 3 weeks of pregnancy in the sow (Taverne *et al.*, 1979b). However, the action of PGE_2 on the pig myometrium is unknown and further information is needed on the plasma concentrations of PGE_2 in the maternal uterine vein and artery during gestation in the pig.

Rice *et al.* (1989) have characterized the gestational profile of PGE_2 release from chorioamnion and chorioallantois in sows between 47 and 112 days' gestation. PGE_2

release from explants of the combined chorioallantois and chorioamnion under basal conditions is low (i.e. 13.7 ± 2.8 pmol/100 mg/h) up to 89 days (0.7 gestation). Release significantly increased by 3.5-fold over the next 22 days and reached a maximum of 176.5 ± 34.9 pmol PGE_2/100 mg/h. The addition of arachidonic acid significantly increased the release of PGE_2 at all stages of gestation, but when expressed as a percentage of basal PGE_2 release this effect decreased with increasing gestational age. Thus, with increasing gestational age, arachidonic acid became less rate limiting, implying an increase in phospholipase activity. However, the marked increase in PGE_2 synthesis in the presence of arachidonic acid between 80 and 110 days indicates a marked increase in PGH synthase activity in the pig placenta. Whether this is due to an increase in the tissue content of the PGH synthase enzyme, an increase in the rate of catalytic conversion or an increase in the half life of the enzyme is still to be determined (Rice *et al.*, 1989). Changes in the maternal plasma pattern and placental production of PGE_2 in the sheep are described in section 9.5. The pig may follow a similar pattern, and if so, PGE_2 may play a luteotrophic role in the pig.

9.4.4 LUTEAL REGRESSION AND HORMONAL CHANGES AT BIRTH

Parturition in the pig is preceded by a complex pattern of hormonal changes (reviews by First and Bosc, 1979; Taverne, 1982). A decrease in P_4 concentrations (Robertson and King, 1974; Ash and Heap, 1975) caused by the regression of the CL plays an essential role in the onset of parturition because P_4 treatment after ovariectomy prevents premature delivery (First and Staigmiller, 1973; Sherwood *et al.*, 1978) and induction of additional CL on days 103 or 108 of pregnancy prolongs gestation (Bosc *et al.*, 1974; Martin *et al.*, 1977). There is some debate about the exact timing of the decrease in P_4 levels and whether it precedes or follows the increase in relaxin concentrations, depending partly on the frequency of sampling and what is interpreted as a significant increase. Watts *et al.* (1988), using hourly sampling for the last 48 h, reported that the first change detected was a decrease in P_4 concentrations, which began at about 30 h prepartum and progressed to term. At about 20 h before delivery, plasma PGFM concentrations increased sharply, rising more slowly thereafter, and there was a surge in plasma relaxin concentrations 15–12 h prepartum, reaching a maximum of 55–329 ng/ml. The maternal plasma concentrations of oestradiol-17β varied within and amongst gilts over a relatively wide range, but in all animals an increase was observed during the last 30 h.

In contrast, Whitely *et al.* (1990) reported that relaxin concentrations increased before P_4 concentrations started to decline, as found by Taverne *et al.* (1982), who considered that maternal plasma prolactin and relaxin concentrations started to rise almost simultaneously between 57 and 47 h before the first piglet and that both hormones reached peak values when the plasma concentrations of P_4 had started to decline rapidly.

There is, however, marked variation between animals in the timing of the relaxin increase and the P_4 decrease. A study of individual sows (Sherwood *et al.*, 1981) showed that relaxin concentrations rose steadily from 2.7 ng/ml on day 110 to 15.8 ng/ml on day 112 and during the 2 days preceding parturition there was a further marked increase, with values reaching a peak 15 h before the birth of the first piglet. During days 110–112 small peaks of relaxin were detected, and these are probably in response to small amounts of $PGF_{2\alpha}$ released from the uterus. $PGF_{2\alpha}$ is known to be released in a pulsatile manner (Gleeson *et al.*, 1974), but these pulses can only be detected satisfactorily by high-frequency sampling from the uterine vein. The measurement of PGFM in peripheral plasma is a relatively insensitive means of

measuring $PGF_{2\alpha}$ release from the uterus. The administration of indomethacin to the preparturient sow not only blocks parturition but also prevents the increase in plasma concentrations of relaxin and prolactin (Taverne *et al.*, 1982), indicating that $PGF_{2\alpha}$ is involved in the triggering process for these changes.

If $PGF_{2\alpha}$ is the luteolysin in the pig, then only very low concentrations of $PGF_{2\alpha}$ would be needed in the arterial blood to initiate luteolysis. The efficacy of $PGF_{2\alpha}$ as a luteolysin is dependent not only on the concentration achieved but also on the time the CL is exposed to the $PGF_{2\alpha}$ the longer the better (Thorburn and Nicol, 1971). The efficacy of the $PGF_{2\alpha}$ analogues relates to their relatively long biological half-life, because they are not metabolized as rapidly by the lungs. A small initial release of $PGF_{2\alpha}$ causes a small decrease in P_4 concentrations, which in turn causes a further release of $PGF_{2\alpha}$. Thus, as the P_4 levels fall, greater amounts of $PGF_{2\alpha}$ are released, causing further decreases in P_4. If a luteolytic dose of a $PGF_{2\alpha}$ analogue (e.g. cloprostenol) is given to sows to induce farrowing, it is some 6 h before the initial increase in endogenous $PGF_{2\alpha}$ release occurs, and the $PGF_{2\alpha}$ concentrations continue to increase over the next 20 h, reaching very high values similar to those found at spontaneous delivery (Silver *et al.*, 1979a, 1983).

Further evidence in favour of $PGF_{2\alpha}$ being the luteolysin in the sow is provided by the studies of Martin *et al.* (1977). When CL were induced in pregnant gilts on day 83 or 103, and the original CL removed 6 days later, pregnancy was maintained. CL induced on day 83 had no effect on the length of gestation, and remained functional for 30 days rather than 15 days, as do the CL of the oestrous cycle, or 114 days, as do the CL of gestation. These results suggest that there was no circulating $PGF_{2\alpha}$ (or other luteolysin) in the pregnant sow before normal term because the CL persisted despite being sensitive to the luteolytic action of $PGF_{2\alpha}$. These observations also show that these CL are responsive to $PGF_{2\alpha}$ because they regress at term. The CL induced on day 102 remained functional for 15 days and prolonged gestation beyond the usual 114 days (to 119 days), exhibiting an intrinsic lifespan as in the oestrous cycle (du Mesnil du Buisson and Léglise, 1963). The CL of the oestrous cycle are resistant to luteolytic doses of $PGF_{2\alpha}$ during the first 12 days of the cycle, and presumably the new CL induced on day 103 are resistant to the parturient luteolysin.

$PGF_{2\alpha}$ treatment causes the regression of the old CL, but CL induced less than 12 days previously are resistant; plasma P_4 levels decreased but not sufficiently to cause parturition (Coggins *et al.*, 1977). $PGF_{2\alpha}$ and its analogues are potent luteolytic agents in the sow, and the fall in maternal plasma P_4 and rise in PGFM follow the same patterns as those seen before spontaneous labour at term (First and Bosc, 1979; Silver *et al.*, 1979a, 1983; Nara and First, 1981). Indomethacin prevents the preparturient increase in PGFM, luteal regression, the decrease in maternal P_4 concentrations and parturition (Nara and First, 1977). In indomethacin-treated sows, exogenous $PGF_{2\alpha}$ caused a decrease in P_4 concentrations and delivery, but very high doses given over long periods were required to increase the concentration of $PGF_{2\alpha}$ metabolites in the blood equal to those found at delivery (Nara and First, 1977). These results again indicate that only very low concentrations of $PGF_{2\alpha}$ are needed to initiate luteal regression at term in the sow.

The luteolytic agent in the sow is of uterine origin because hysterectomy at day 112 prolongs the life of old CL and new CL that have been induced about 7 days before the expected term (Bosc *et al.*, 1974; First and Bosc, 1979). The luteolysin travels from the uterus to the ovaries, at least in part by the systemic route. Parturition occurs at the expected time after ovaries are transplanted to the uterus or the body wall, thus surgically disrupting the normal anatomical relationship between the uterus and ovaries and pre-

venting any countercurrent transfer of the luteolysin between the uterine vein and ovarian arteries (Martin *et al.*, 1978). If $PGF_{2\alpha}$ is the luteolysin, these results would suggest that the lungs of the pregnant pig are not fully effective in clearing $PGF_{2\alpha}$ from the blood during its passage through the lungs.

Maternal plasma P_4 concentrations decrease slowly over the last 10–14 days of gestation before decreasing rapidly over the last 24–36 h associated with luteal regression (Robertson and King, 1974) caused by withdrawal of trophic support during the last 2 weeks of gestation or release of very small amounts of the $PGF_{2\alpha}$. Whatever the cause, the gradual decrease in P_4 concentrations over the last 10 days may also initiate the release of small amounts of $PGF_{2\alpha}$, which in turn initiate the final luteolytic mechanism.

9.4.5 THE EVOLUTION OF MYOMETRIAL ACTIVITY BEFORE PARTURITION

During the last 3 weeks of gestation, when circulating concentrations of P_4 are relatively high, myoelectric activity in the uterus is minimal and restricted to episodes of several minutes' duration, principally in areas of muscle overlying the fetuses (Taverne *et al.*, 1979b). Uterine EMGs consist of irregular episodes (<3.5/h) of prolonged phases of activity (>3.5 min duration) in uterine segments containing a fetus. Empty parts of the uterus remain relatively inactive (Taverne *et al.*, 1979b). Plasma oxytocin concentrations remain below 1.2 μu/ml between 12 and 2 days before parturition (Forsling *et al.*, 1979). Between 24 and 10 h before expulsion of the first piglet, mean concentrations of P_4 significantly fall and those of oestrogens increase. This coincides with behavioural changes of the sow (nest building), but myometrial activity at this stage is similar to that recorded on the previous days (Taverne *et al.*, 1979b). It was only between 9 and 4 h before the first piglet is born that the pattern of the EMGs changes and regular phases of electrical discharges appear with increasing frequency (7–12/h in all parts of the uterus). At the same time, oxytocin concentrations in peripheral plasma are elevated. During delivery of the piglets the mean frequency of the phases of electrical discharges increases further (18–20/h, their duration being significantly shorter than in the previous few hours). Oxytocin concentrations reach their highest levels at this stage, oestrogen concentrations are already falling and P_4 is <4 ng/ml in all sows (Taverne *et al.*, 1979b). Watts *et al.* (1988) found that total EMG activity did not change between 20 and 10 h prepartum, despite a marked increase in circulating PGFM concentrations, but in ovariectomized gilts, which therefore lacked relaxin, EMG activity increased steadily from about 15 h after the last P_4 injection when plasma P_4 titres were falling below 40 ng/ml. Watts *et al.* (1988) suggested that the difference between these two groups might be the sharp increase in plasma relaxin titres which occurred in control animals as P_4 concentrations waned. Consistent with this view was their finding that the progressive rise in EMG activity observed in the ovariectomized gilts after the last P_4 injection was virtually abolished in another group of animals by the administration of relaxin. These studies agreed with those of Porter and Watts (1986), who suggested that relaxin appears to maintain myometrial quiescence as the P_4 block wanes. When relaxin concentrations decrease, EMG activity increases progressively up to birth, as reflected by significant negative correlation between relaxin and mean total duration of electrical activity, suggesting that relaxin inhibits the myometrium while P_4 titres are falling and while circulating levels of $PGF_{2\alpha}$ are increasing (Watts *et al.*, 1988), as found for rats (Porter *et al.*, 1979).

Progesterone is a potent inhibitor of myometrial activity in the ovariectomized nonpregnant pig, and the ability of P_4, or synthetic progestagens (Craig, 1982) to maintain pregnancy in the sow after bilateral ovari-

ectomy, at least in part, may be due to inhibition of myometrial contractions by P_4 (Porter and Watts, 1986). As in rabbits (Csapo *et al.*, 1963) and sheep (Lye and Porter, 1978), the effect of P_4 is gradual in onset, the reduction in the frequency of pressure cycles occurring progressively over a period of 6–24 h. In contrast to relaxin, P_4 abolishes the myometrial responsiveness to oxytocin, and at least partial withdrawal of P_4 is a prerequisite for parturition (Taverne, 1982).

9.4.6 RELAXIN

The CL of pregnant pigs synthesize relaxin and store it in membrane-bound vesicles. The content of relaxin in the CL increases from about day 28, reaching peak levels at day 110 of gestation and, then decrease (Belt *et al.*, 1971; L.L. Anderson *et al.*, 1983), markedly so between 44 and 26 h before parturition when relaxin is discharged into the blood (Belt *et al.*, 1971). Plasma concentrations are markedly elevated only during the 2 days before parturition: peak values (50–300 ng/ml) occur 14–22.5 h before parturition, and concentrations then decrease rapidly to low levels (1 ng/ml) by 1 day after farrowing (Sherwood *et al.*, 1975, 1981). The high levels of relaxin in plasma are associated with high plasma concentrations of PGFM and the regression of the CL (Sherwood *et al.*, 1979; Nara and First, 1981; Taverne *et al.*, 1982; Watts *et al.*, 1988). It is thought that $PGF_{2\alpha}$ acts on the CL to cause the secretion of relaxin, concentrations increasing sharply after a luteolytic dose of exogenous $PGF_{2\alpha}$ (Sherwood *et al.*, 1976, 1979). Moreover, relaxin concentrations peaked 10 min after a small dose of $PGF_{2\alpha}$ (50 μg i.v.) which did not cause luteolysis and was not sufficient to decrease maternal P_4 concentrations (Nara *et al.*, 1982). Inhibition of PG synthesis by indomethacin prevented the release of relaxin, while simultaneous treatment with exogenous $PGF_{2\alpha}$ induced release of relaxin from the CL (Nara and First, 1981). Before parturition in the sow, maternal plasma prolactin and relaxin concentrations start to increase at about the same time (Taverne *et al.*, 1982). Although a common stimulus may evoke the relaxin and prolactin spikes, they are independent because abolition of the prolactin spike with bromocriptine did not prevent the occurrence of the relaxin spike (Taverne *et al.*, 1982). In bromocriptine-treated sows, normal preparturient changes in relaxin and P_4 were observed and parturition was unaffected, indicating that increasing prolactin concentrations were not an essential component of parturition. The prepartum changes in plasma prolactin, relaxin and P_4 concentrations were prevented by indomethacin but occurred apparently unchanged after the treatment ended, suggesting a primary role for $PGF_{2\alpha}$ in the prepartum hormone changes and the initiation of parturition.

The significance of the prepartum increase in relaxin concentrations is still uncertain, but it may be involved in the softening of the cervix (Kertiles and Anderson, 1979) and in myometrial coordination (Downing *et al.*, 1980). The main role of relaxin in the pig would appear to be as a myometrial inhibitor (Porter and Watts, 1986; Watts *et al.*, 1988).

Relaxin appears to maintain uterine quiescence as the P_4 block (Csapo, 1956) wanes. Porter and Watts (1986) demonstrated that P_4 is a potent inhibitor of myometrial activity in the ovariectomized non-pregnant pig, and suggested that the ability of P_4, or synthetic progestagens (Craig, 1982), to maintain pregnancy in the sow after bilateral ovariectomy, at least in part, is due to its inhibition of myometrial contractions. They found that, as in the rabbit (Csapo *et al.*, 1963) and the ewe (Lye and Porter, 1978), the effect of P_4 was gradual in onset since the reduction in the frequency of pressure cycles occurred progressively over a period of 6–24 h. In contrast to relaxin, P_4 abolished the myometrial responsiveness to oxytocin. Porter and Watts (1986) concluded that the pig is like the rabbit (Csapo *et al.*, 1963) and sheep (Lye and

Porter, 1978), in that a classic P_4 block 'can be demonstrated, a conclusion which is consistent with the reports that at least partial withdrawal of the action of the steroid is a prerequisite for parturition' (Taverne, 1982).

Purified porcine relaxin is a powerful myometrial inhibitor in the pig *in vivo* (Porter and Watts, 1986). Relaxin lowers the frequency of intrauterine pressure cycles without reducing the amplitude. A single dose of relaxin that produces plasma concentrations similar to those found normally prepartum is able to reduce myometrial activity almost to zero. Relaxin inhibition of the uterus in pigs does not affect the responsiveness of that organ to oxytocin (Porter and Watts, 1986). It appears that (1) relaxin is able to inhibit myometrial contractility of the gravid pig uterus *in vivo* and (2) the evolution of labour activity in the sow is inversely correlated with plasma relaxin concentrations (Porter, 1979; Porter and Watts, 1986; Watts *et al.*, 1988).

9.4.7 MATURATION OF THE FETAL PITUITARY–ADRENAL AXIS

In the pig, fetal adrenal weight increases during late gestation (Dvorak, 1972) and plasma cortisol concentrations in chronically catheterized fetuses increase during the last 4–9 days of gestation (Silver *et al.*, 1979a; Randall, 1983; Silver and Fowden, 1989). Therefore, activation of the fetal pituitary–adrenal axis presumably initiates parturition in pigs in a way similar to that in ruminants (Liggins *et al.*, 1973; Thorburn and Challis, 1979). Decapitation (Stryker and Dziuk, 1975) or hypophysectomy (Bosc *et al.*, 1974; Kendall *et al.*, 1983; Randall and Tsang, 1986) of the entire litter at an early stage of pregnancy results in a prolongation of pregnancy and eventual death of the fetuses *in utero*, emphasizing the importance of the fetal pituitary (and the brain) in the initiation of labour in pigs. When the litter contained one intact fetus plus three or four headless fetuses, parturition was delayed to day 124 or 125. When more than one intact fetus was present in a litter containing some headless fetuses, parturition was not delayed and two intact fetuses were needed to induce labour at term (Stryker and Dziuk, 1975). However, these studies did not point specifically to ACTH and the hypothalamic–pituitary–adrenal axis.

A single transuterine injection of a large dose of long-acting ACTH to each fetus, or all except two, at 103 days' gestation marginally shortened gestation to 110–112 days compared with controls, 113–114 days (Bosc, 1973). Dexamethasone (100 mg/day) administered to pregnant sows on days 101–104 also shortened the length of gestation to 110.5 days as compared with 115 days in controls (Coggins and First, 1977). A similar delay between injection of dexamethasone (75 mg/day on days 101–103) to the pregnant sow and the time of delivery (112.6 ± 0.6 days compared with 114.7 ± 0.2 days) was observed by North *et al.* (1973). Dexamethasone (6 mg) administered to seven fetuses of a litter also resulted in premature delivery some 7–8 days later (North *et al.*, 1973).

Although these findings are consistent with the suggestion that the initiation of parturition may be actively triggered by the fetal pituitary–adrenal axis (First *et al.*, 1982), this conclusion has been criticized (Silver and Fowden, 1989; Randall *et al.*, 1990). Randall *et al.* (1990) consider that these experiments are inconclusive because large doses were used and were superimposed on the stress response associated with surgical procedures. Furthermore, studies in ruminants have shown that when dexamethasone is used in pharmacological doses to induce premature parturition, the increase in placental 17α-hydroxylase activity occurs rapidly in the first 12–24 h and parturition occurs within 48–72 h (Thorburn and Challis, 1979). The delay observed in the pig experiments suggests that dexamethasone may be working by a mechanism other than a direct action on placental 17α-hydroxylase. Studies in pregnant cows have shown that if dexamethasone

(possibly because the dose was inadequate) does not induce parturition within a few days, it still initiates premature parturition, but delivery occurs some 7–10 days later (Fairclough *et al.*, 1981). However, studies in sheep suggest that glucocorticoid treatment may increase the responsiveness of the fetal hypothalamic–pituitary–adrenal axis or act at some other site(s) to provoke another parturient mechanism independent of the fetal pituitary–adrenal axis (Liggins *et al.*, 1977). There is some compelling evidence to indicate that the fetal pituitary–adrenal system is not directly involved in initiating parturition in the pregnant sow.

Early hypophysectomy by coagulation of the fetal pituitary between days 68–84, selectively alters the growth and maturation of individual organs. Hypophysectomy (before day 70) resulted in a significant reduction in the weights of the thyroid, adrenal and the testes but the spleen and kidneys increased (Randall, 1989). Differences occur between fetal lambs hypophysectomized late (115–125 days' gestation) and early (70–75 days' gestation) (Deayton *et al.*, 1993). Ewes carrying fetuses hypophysectomized earlier (days 70–75) failed to show the normal increase in placental oestrogen and PGE_2 biosynthesis between days 90 and 125 (Deayton *et al.*, 1993). They suggested that the fetal pituitary, perhaps by the synthesis and release of gonadotrophins, may influence placental steroid and PGE_2 synthesis. It is not known whether fetal infusion of dexamethasone or ACTH will induce premature labour in ewes carrying fetuses hypophysectomized early in gestation (day 75 or earlier). It is possible, therefore, that in pigs, in which the fetuses have been decapitated or hypophysectomized early in gestation (Stryker and Dziuk, 1975; Coggins and First, 1977), the maturation of the placenta and other key organ systems may also be delayed (Randall, 1989). This suggestion is consistent with the observation of the inability of dexamethasone to induce delivery in sows carrying decapitated fetuses (Coggins and First, 1977) and its failure to reduce maternal P_4 concentrations (Coggins *et al.*, 1977).

The parturition-inducing ability of glucocorticoids increases with advancing gestation in a number of species (Adams and Wagner, 1970; Bosc, 1972; Coggins and First, 1973) and may be dependent on a maturational process also controlled by the fetal pituitary or at least some portion of the fetal brain (Coggins and First, 1977). Furthermore, in the sow, as in the sheep (Chapter 10, this volume), fetal hypophysectomy (or decapitation) prevents the increase in placental PGE_2 production during the last 0.3 of gestation, and so the lack of PGE_2 may interfere with parturient mechanisms.

Randall *et al.* (1990) measured the fetal and maternal hormone changes in plasma from gilts in which all fetuses were infused with ACTH (2 μg/h) or saline continuously from day 103 of gestation. Litter size had been reduced to four earlier in gestation. The fetal adrenals were significantly heavier in fetuses receiving ACTH, and their plasma cortisol values increased rapidly in response to the ACTH infusion, although there was a marked variation in individual piglets. The fetal plasma cortisol concentrations were similar to those seen in the last few days of gestation (Silver *et al.*, 1979a; Randall, 1983). The fetal adrenal is fully responsive *in vitro* to ACTH by about 90 days' gestation (Lohse and First, 1981). A rise in fetal plasma cortisol in response to ACTH can be elicited by fetal manipulation as early as 70 days (0.65 gestation): infusion of ACTH (125 μg/day), over a 4-day period, elicited a cortisol response (Silver and Fowden, 1989) similar to that observed by Randall *et al.* (1990).

In chronically catheterized fetuses, Silver and Fowden (1989) noted a gradual rise in both cortisol and ACTH in fetal plasma from day 100 to term. There was a significant correlation between plasma cortisol and log plasma ACTH values ($r=0.81$, $P<0.001$). Analysis of basal values from younger (day

70–100) fetuses showed a positive correlation between cortisol and log ACTH, but the slope of the regression line was significantly less than for the older fetuses, indicating a greater adrenocortical response to a given level of ACTH nearer term.

In neither of the two studies in which ACTH was infused into chronically catheterized fetuses was premature parturition induced. In the experiments of Randall *et al.* (1990), all fetuses were infused with ACTH (2 μg/h) from day 103, whereas in the study of Silver and Fowden (1989), a continuous infusion of ACTH (0.125 mg/day) for 4–5 days was given to only 1–2 fetuses per litter (total size 7–12). Both studies caused an increase in fetal cortisol to parturient levels. It is difficult to reconcile these findings with the earlier studies, which suggested that activation of the fetal pituitary–adrenal axis initiated parturition in the pig (First and Bosc, 1979; First *et al* 1982).

These results indicate that the fetal pituitary axis is not involved in parturition *per se*, but that it probably plays a very important role in the maturation of the fetal lung and many other systems essential for post-natal survival of the neonate. It seems unlikely that the same mechanism is operative in the sow as in the goat since, in the goat, oestrogen is luteolytic (Currie and Thorburn, 1976), whereas in the sow, oestrogen is luteotrophic (Flint *et al.*, 1979).

In sheep and goats, the main action of glucocorticoids is to increase oestrogen biosynthesis by increasing placental 17α-hydroxylase activity (A.B.M. Anderson *et al.*, 1975; Flint *et al.*, 1978). Increased placental production of oestrogens would serve little purpose in sows because oestrogens are not luteolytic (Flint *et al.*, 1979). It appears that some other mechanism is involved in stimulating $PGF_{2\alpha}$ release from the pig placenta, if $PGF_{2\alpha}$ is the likely luteolysin in the pig.

Pituitary factors (e.g. gonadotrophins) may be required for the placental production of PGs in sows (Silver *et al.*, 1979b). Methallibure, an inhibitor of hypothalamic function (Mueh *et al.*, 1975) and specifically LH release, causes prolonged pregnancy when administered to the pregnant sow (First, 1972; Coggins and First, 1977). The fetal pituitary may therefore be responsible (Rice *et al.*, 1989), as in the sheep (Thorburn *et al.*, 1989; Deayton *et al.*, 1993), for a progressive increase in PGH synthase (cyclooxygenase) activity in the placenta but the final trigger may depend on the massive release of substrate (arachidonic acid) for $PGF_{2\alpha}$ synthesis and the presence of the enzyme ($PGF_{2\alpha}$ isomerase) which converts PG endoperoxides to $PGF_{2\alpha}$, or the enzyme 9 keto-reductase, which converts PGE_2 to $PGF_{2\alpha}$. The nature of this trigger is unknown.

9.4.8 OESTROGENS

Oestrogen concentrations increase in maternal (Robertson and King, 1974) and fetal (A.A. Macdonald *et al.*, 1979) circulations during the last month of gestation, with a more rapid increase during the last week. The rapid decrease after parturition suggests that oestrogens are produced by the placenta (Fèvre *et al.*, 1968; Robertson and King, 1974), supported by the finding that negative arteriovenous differences for conjugated oestrone and oestradiol-17β exist across both the uterine and umbilical circulations (Silver *et al.*, 1979a; Randall and Tsang, 1986). The high oestrogen content of the placental tissue (Choong and Raeside, 1974) and the demonstration that fetal placental tissue can convert androstenedione or P_5 to oestrogens (Ainsworth and Ryan, 1966; Craig, 1982) are additional evidence for the placental synthesis of oestrogens.

Progesterone concentrations are significantly higher and oestrogen concentrations significantly lower in hypophysectomized fetuses (Kendall *et al.*, 1985; Randall and Tsang, 1986) than in intact fetuses. When fetuses in one uterine horn only were hypophysectomized, oestrogen concentrations in

the uterine vein draining the horn containing intact fetuses were higher than those from the contralateral vein (Randall and Tsang, 1986), indicating that fetal and maternal oestrogen concentrations are influenced by the fetal pituitary. These observations are consistent with the earlier findings in sheep (Kendall *et al.*, 1977) in which fetal hypophysectomy also resulted in low fetal and maternal oestrogen concentrations and which have been recently confirmed (Thorburn *et al.*, 1989; Deayton *et al.*, 1993).

In the absence of the fetal pituitary, the placenta still produced a small amount of oestrogens since a negative arteriovenous difference still existed between the umbilical vessels (Randall and Tsang, 1986). The nature of the pituitary factor(s) is unknown. The higher fetal P_4 concentrations in the hypophysectomized pig fetuses suggest that P_4 is not being metabolized to oestrogens and that there is a low level of 17α-hydroxylase in the placenta of the hypophysectomized fetus. This would account for the low oestrogen levels found in these sows and their fetuses (Randall and Tsang, 1986). The high oestrone concentrations in hypophysectomized fetuses after surgery were thought to be due to some other steroid (possibly androstenedione derived from the adrenals) being converted to oestrogens.

The fetal placenta of the pig has the ability to convert P_4 to oestrogens and 17α-hydroxylase increases between days 106 and 114 of gestation (Craig, 1982), a period when fetal cortisol concentrations are rising (Silver *et al.*, 1979a; Randall, 1983; Silver and Fowden, 1989; Randall *et al.*, 1990). Craig (1982) suggested that, as in sheep and goats (Thorburn and Challis, 1979), fetal cortisol may be responsible for the increase in 17α-hydroxylase. However, infusion of dexamethasone into hypophysectomized fetuses for 96 h did not cause significant alterations in P_4 or oestrogen concentrations (Randall and Tsang, 1986), despite the concentration infused being higher than that previously used to suppress endogenous cortisol production in intact fetuses (Randall *et al.*, 1984). When ACTH or dexamethasone was infused into intact fetuses oestrone concentrations did not increase markedly but P_4 concentrations decreased after about 6 days of infusion (Randall and Tsang, 1986). No consistent changes were seen in the maternal oestrogen or P_4 concentrations during the course of ACTH infusions into all fetuses, but fetal plasma P_4 values fell during the infusion both of ACTH and saline (Randall *et al.*, 1990). The available data would suggest that, if fetal cortisol does increase 17α-hydroxylase activity in the porcine placenta, the increase is not great and its action on fetal and maternal steroid levels is also not significant. The need for oestrogen in the pig seems more obscure since oestrogens are not luteolytic in the pregnant sow (Flint *et al.*, 1979). Oestrogens may play a less obligatory role by raising oxytocin receptor concentrations in the placenta, endometrium and myometrium.

9.4.9 OXYTOCIN

In the pig, plasma oestrogen concentrations increase slowly over the last 3 weeks before parturition to reach peak values at delivery. In other species, oestrogens are known to stimulate the release of oxytocin (Roberts and Share, 1969; Roberts, 1975) and increase the sensitivity of the uterus to the hormone (Theobald, 1970), and myometrial sensitivity to oxytocin might therefore be enhanced by oestrogens during the last 3 weeks of pregnancy. This increased sensitivity may be due to an increase in the number of oxytocin binding sites (Soloff, 1975) present in the pig uterus (Soloff and Swartz, 1974). Myometrial activity increases and continues until completion of delivery; this coincides with elevated concentration(s) of oxytocin in the maternal plasma (Forsling *et al.*, 1979; Taverne *et al.*, 1979b). During delivery of the piglets the frequency of contractions in-

creases and oxytocin concentrations reach their highest values (Taverne *et al.*, 1979b), when oestrogen concentrations are already falling and P_4 has reached values below 4 ng/ml. Forsling *et al.* (1979) concluded that the only steroid that seemed to relate to oxytocin release was the negative correlation with P_4. Taverne *et al.* (1979b) noted with some reservations that the raised oxytocin concentration may precipitate the rather sudden onset of the regular pattern of uterine activity observed between 9 and 4 h before delivery of the first piglet.

There is a massive release of $PGF_{2\alpha}$ from the pregnant uterus during labour (Silver *et al.*, 1979a). Since $PGF_{2\alpha}$ stimulates the release of oxytocin in the sow (Ellendorff *et al.*, 1979), there is a possibility that $PGF_{2\alpha}$ and oxytocin may exhibit a positive-feedback effect on their own release (Thorburn *et al.*, 1977a,b; Ellendorff *et al.*, 1979), leading to the evolution of myometrial activity adequate to ensure safe delivery of the piglets and their placentas.

9.4.10 SUMMARY

The maintenance of pregnancy in the sow depends on the continued function of the CL and its ability to secrete P_4. During the second half of pregnancy trophic support for the CL seems to depend on prolactin (and to a lesser extent LH) from the maternal pituitary, but there appears to be no decrease in plasma concentrations of prolactin to account for the decrease in P_4 concentrations during the last 10–14 days before delivery.

We propose that PGE_2, derived from the placenta, may be luteotrophic during late pregnancy, but information is lacking on the plasma concentrations of PGE_2 in the pig and whether it is luteotrophic to the late pregnant pig CL. Oestrogens secreted by the placenta may provide a further luteotrophic stimulus to the CL. The relative importance of these factors needs to be evaluated. It is possible that one or more of these substances may act indirectly to suppress the release of $PGF_{2\alpha}$, the ultimate luteolysin in the sow.

The gradual decrease in P_4 concentrations during the 10–14 days before delivery suggests that there is a gradual decrease in the trophic support for the CL. If the fetus(es) is to play a primary role in the initiation of parturition in this species, it would seem that some mechanism must exist for the fetus(es) to switch off this trophic support. Maternal pituitary hormones such as prolactin and LH are unlikely to be involved. There is no evidence of a decrease in oestrogen secretion by the placenta. A switch in the synthetic pathway for PGs from E_2 to $F_{2\alpha}$, that is from a luteotrophin to luteolysin, would seem an effective mechanism which theoretically might be achieved by the fetus.

As in the sheep, PGE_2 may be synthesized in the fetal placenta and membranes and $PGF_{2\alpha}$ produced by the endometrium and maternal placenta, with oxytocin stimulating the secretion of $PGF_{2\alpha}$ but not PGE_2. The chorion (trophoblast) may secrete a substance which inhibits $PGF_{2\alpha}$ synthesis in the maternal endometrium and placenta, the inhibition being directed at the phospholipase or PGH synthase steps of PG synthesis, or both. This inhibition could be switched off at an appropriate time by the fetus increasing $PGF_{2\alpha}$ release and initiating luteal regression.

The present data strongly indicate that $PGF_{2\alpha}$ is the luteolysin that precipitates CL regression and parturition in the sow. Some days before delivery small amounts of $PGF_{2\alpha}$ are released from the uterus and act on the CL to cause the initial small pulses of relaxin and to initiate regression of the CL. The initial decrease in P_4 concentrations enhances the release of $PGF_{2\alpha}$ which, in turn, acts back on the CL to decrease P_4 release further and stimulate relaxin release. Thus, the interaction of $PGF_{2\alpha}$ and P_4 forms a positive feedback (Figure 9.5).

In the parturient sow, increasing relaxin and oestrogen concentrations and decreasing P_4 values may stimulate the release of oxy-

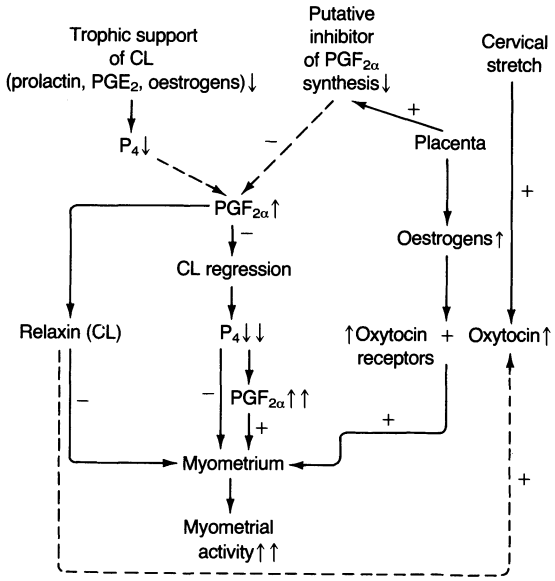

Figure 9.5 The pathways involved in the initiation of parturition in the pig.

tocin from the posterior pituitary. With the birth of the first piglet, the Ferguson reflex would stimulate further oxytocin release. Preliminary data indicate that significant levels of PGE_2 escape metabolism in the lungs and appear in the arterial circulation and may account for the increase in uterine activity during the last three weeks of pregnancy in the sow. With the decrease in P_4 concentrations, the high arterial levels of PGE_2 may account in part for the uterine activity at parturition. It is known that PGE_2 can act via either the IP_3 or the adenylate cyclase pathway, and theoretically could stimulate or inhibit uterine activity dependent on the nature of the PGE_2 receptors present on the myometrium at any time. However, PGF_α has to be transported by the systemic route to cause CL regression in the pig, and it is more likely that in the pig, as in other species, $PGF_{2\alpha}$ synthesized in the endometrium and maternal placenta may diffuse directly into the myometrium to stimulate activity. Only small amounts of PGs may be required to stimulate myometrial activity as P_4 values decrease. The increase in oestrogen concentrations before parturition is probably an expression of increased activity of the fetal pituitary–adrenal axis. Oestrogens increase oxytocin release and increase the number of oxytocin receptors in the endometrium, myometrium and placenta, and thus facilitate the release of PGs and the direct action of oxytocin.

9.5 SHEEP

9.5.1 INTRODUCTION

Most research in the past 25 years on the mechanism of parturition has been on the sheep. There are many reasons for this: sheep are important economically in many countries and farmers and governments actively contribute to research on them; sheep withstand surgery well and are therefore excellent experimental models in pregnancy, allowing the development of new techniques for maintaining vascular catheters in the fetus and sophisticated surgical techniques, such as hypophysectomy, pituitary stalk section, thyroidectomy, bilateral nephrectomy and hypothalamic–pituitary disconnections. The relatively large size of the section on the sheep testifies to the scope of the literature on parturition in this species and the importance of these studies.

The sheep, like the human, is a species in which the placenta is a major site of P_4 production. In this respect, the sheep contrasts with the goat and cow and most other ruminant species (Heap *et al.*, 1977) in which the CL constitutes the major site of P_4 synthesis. In all ruminants, P_4 exerts a profound inhibitory influence on myometrial activity. The initiation of parturition in these species is mainly directed at removing this inhibitory action of P_4. It is instructive to compare the mechanisms by which the fetus initiates its own delivery by inhibiting P_4 secretion,

whether this P_4 is produced in the placenta or CL (Figure 9.6).

9.5.2 PROGESTERONE

P_4 is essential for the maintenance of pregnancy in sheep. During the first half of pregnancy, the concentration of P_4 gradually increases from luteal phase levels, but at approximately day 90 its concentration increases markedly, reaching a peak at about day 125 of gestation (Basset *et al.*, 1969; Thorburn *et al.*, 1977a), before decreasing in the last few days before parturition at 145 days (Bassett *et al.*, 1969; Stabenfeldt *et al.*, 1972).

(a) Site of production

The CL is the major site of P_4 production during the first third of pregnancy, ovariectomy of the ewe before day 50 of pregnancy resulting in abortion (Casida and Warwick, 1945; Denamur and Martinet, 1955). The *in vitro* studies of R.V. Short and Moore (1959) and Ainsworth and Ryan (1967) suggested that the ovine placenta was the site of P_4 synthesis during late pregnancy. This was later confirmed by Linzell and Heap (1968),

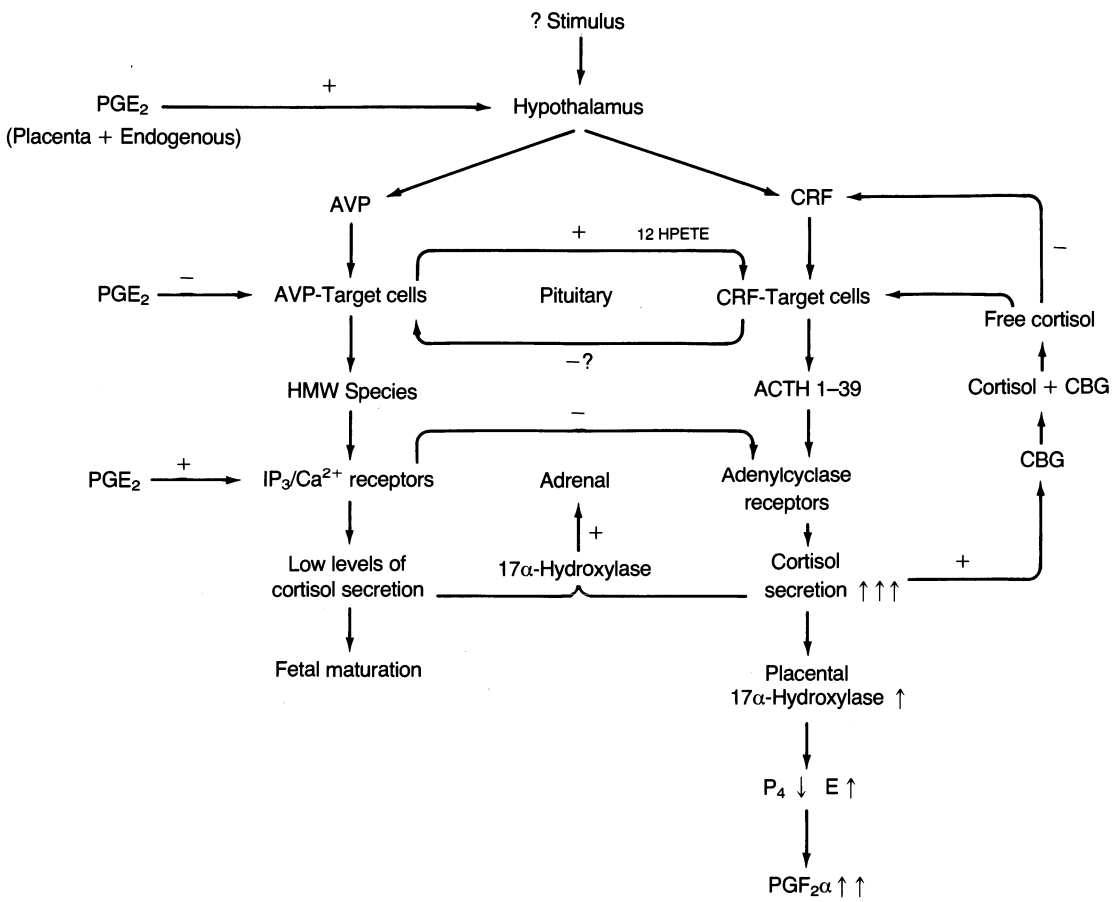

Figure 9.6 The pathways involved in the initiation of parturition in the sheep.

who monitored uterine venous blood concentrations of P_4 in late pregnant ewes. Thorburn *et al.* (1977a) measured plasma concentrations of P_4 in the utero-ovarian vein after ovariectomy on day 55–60: P_4 concentrations decreased to low levels (0.5–1.0 ng/ml), but were sufficient to maintain the pregnancy. From days 55 to 90, there was a progressive increase in P_4 levels in the utero-ovarian plasma, indicating placental P_4 production which was markedly decreased in the few days before delivery. The extra-ovarian contributions to P_4 levels in the circulation have also been measured in ovariectomized ewes in which pregnancy was maintained by treatment with medroxyprogesterone acetate (Ricketts and Flint, 1980): placental production of P_4 rose initially between 50 and 70 days of gestation, with a second phase of increase between 90 and 120 days of gestation, thus confirming the earlier results.

(b) Biosynthesis

Although information is not yet available, it seems likely that in sheep the increase in placental P_4 production between days 90 and 125 of gestation is associated with the placental uptake of lipoprotein from the maternal circulation, as occurs in man (Van Leusden and Villee, 1965; Hellig *et al.*, 1970; Cummings *et al.*, 1982; Simpson, 1984). Lipoproteins, particularly high-density lipoprotein (HDL), stimulated P_4 production in ovine luteal cells in a dose-dependent manner (Diskin *et al.*, 1989) and HDL from the maternal circulation may be the major source of cholesterol for P_4 synthesis. Confirming the results of Flint *et al.* (1974a), Battista *et al.* (1989) showed, using short-term incubation of placental explants from ewes of 133–138 days' gestation, that P_4 synthesis was stimulated by catecholamines via β-adrenoreceptors and adenylate cyclase. LH and the P_4 precursor 25-hydroxycholesterol failed to stimulate basal or agonist-stimulated P_4 synthesis, consistent with the *in vivo* data of J.S. Robinson *et al.* (1977), who showed that fetal hypophysectomy after day 125 of gestation did not affect maternal plasma concentrations of P_4.

In isolated ovine binucleate cells (BNCs), PGE_2, but not LH or cAMP, stimulated P_4 secretion, suggesting that PGE_2 acts through a cAMP-independent mechanism, e.g. the inositol triphosphate (IP_3)/protein kinase C pathway (Wango *et al.*, 1988). The ovine placenta secretes increasing amounts of PGE_2 during the last third of gestation (Fowden *et al.*, 1987), and maternal plasma PGE_2 and P_4 concentrations increase in parallel (Thorburn *et al.*, 1989), but BNCs are not the site of PG synthesis in the ovine placenta. Boshier *et al.* (1991), using immunohistochemical techniques, showed that BNCs did not contain PGHS and uninucleate cells of the trophoblast and the fibroblasts of the ovine placentome in culture did not produce PGs (X. Ralph, unpublished data) (Figure 9.7).

These studies suggest that, once P_4 production is fully established in the ovine placenta (at about 120 days' gestation), P_4 synthesis can be maintained by the combined action of the uninucleate and binucleate cells and that PGE_2, produced in the uninucleate cells via a cAMP-dependent mechanism, acts on the BNCs via a cAMP-independent pathway to stimulate P_4 synthesis. It is possible that, once the HDL receptors and the steroidogenic enzymes are present, they require no further trophic support.

(c) Ovine placental lactogen

There is a parallel increase in maternal plasma concentrations of P_4 and ovine placental lactogen (oPL) (J.S.D. Chan *et al.*, 1978; Taylor *et al.*, 1983). Apoprotein constituents of HDL stimulate a dose-dependent increase in the release of placental lactogen from human trophoblast explants and trophoblast cells (Handwerger *et al.*, 1987), and in pregnant ewes HDL, but not lipoprotein-free

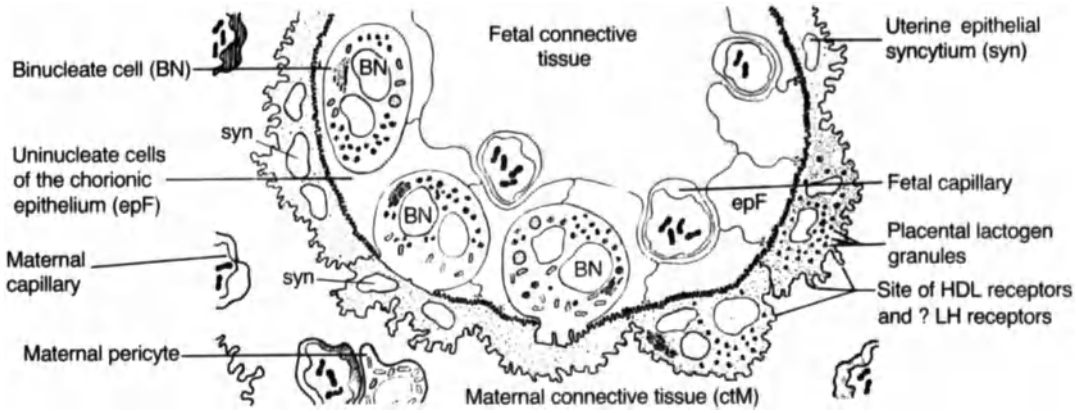

Figure 9.7 The ovine placenta. (Modified from Steven, 1977.)

plasma proteins, stimulates an increase in plasma oPL (Grandis *et al.*, 1989).

Battista *et al.* (1989) reported that oPL secretion was stimulated via the protein kinase C pathway. Since HDL stimulates the release of oPL (Grandis *et al.*, 1989) and oPL is derived from the BNCs (Wooding, 1981), this suggests that there are specific receptors for HDL on the BNCs and that these receptors act via the protein kinase pathway to release oPL. If HDL receptors are present on the maternal surface of the BNCs, it would facilitate delivery of maternal cholesterol directly into the BNCs as substrate for P_4 biosynthesis. Furthermore, the parallel increase in maternal plasma concentrations of P_4 and oPL between 90 and 125 days of gestation would be explained by an increase in HDL receptors on the BNCs. Indeed, as in the human, the major determinant of placental P_4 synthesis may be the number of HDL receptors (Figure 9.8).

The factor(s) that increases the putative lipoprotein receptors on the ovine BNCs is unknown. It has been suggested that hCG/LH may be responsible for inducing low-density lipoprotein (LDL) binding sites in the human CL and thus increase P_4 secretion and that adrenocorticotrophic hormone (ACTH) induces LDL receptors in bovine adrenal cells (Ohashi *et al.*, 1982). It is possible, therefore, that LH may be responsible for the increase in placental P_4 synthesis between 90 and 125 days of gestation by inducing placental lipoprotein receptors and thereby increasing cholesterol delivery to the placenta for P_4 biosynthesis.

(d) Hormonal regulation of placental synthesis

It is not clear whether LH or any other trophic hormone is needed for placental P_4 biosynthesis in the sheep, although LH receptors are present in the placenta. Fetal hypophysectomy at day 100–130 of gestation did not reduce maternal P_4 concentrations, which remained high (Kendall *et al.*, 1977; J.S. Robinson *et al.*, 1977; Ricketts *et al.*, 1980), and it was concluded that P_4 production by the placenta was independent of fetal pituitary control. The *in vitro* studies of Battista *et al.* (1989) and Wango *et al.* (1988) are consistent with that view and suggest that, once P_4 synthesis is established in the placenta, LH is not needed for the maintenance of P_4 synthesis.

Thorburn *et al.* (1989) proposed that fetal pituitary hormones may be needed to initiate the increase in placental steroidogenesis between days 90 and 125 of gestation. After hypophysectomy of fetal lambs between 70 and 75 days of gestation, the increase in P_4

concentrations in maternal plasma between days 90 and 125 of gestation was not significantly affected, but the predicted increase in the plasma concentrations of oestrone sulphate and PGE_2 did not occur (Deayton *et al.*, 1993). These results suggest that fetal pituitary hormones are not essential for the increase in P_4 production but that they are for oestrogen and PGE_2 synthesis by the placenta. If LH is required, an endogenous LH-like substance (?ovine CG) may substitute for fetal pituitary LH. However, if the major determinant of the increase in placental P_4 production is the delivery of cholesterol to the placenta, rather than the regulation of steroidogenic enzymes, then the regulatory factor may be the number of lipoprotein receptors. It is possible, therefore, that HDL and LH receptors are present on the maternal surface of the syncytium (formed from the BNCs) and are exposed to HDL and LH in the maternal circulation. Although maternal hypophysectomy did not affect the normal maintenance of pregnancy (Denamur and Martinet, 1961), P_4 concentrations were not measured.

It is not yet known how the LH receptors are induced, but oPL may be involved: J.S. Chan *et al.* (1980) have shown that oPL can maintain LH receptors in the ovary of the pseudopregnant rat.

A working model may help explain the close relationship between placental oPL and P_4 production. This model predicts that the HDL and LH receptors are both present in the maternal syncytium (derived from BNCs). It suggests that LH of maternal origin may be needed but that it may be replaced by oCG. It is interesting to speculate whether a characteristic of the placental P_4-producing

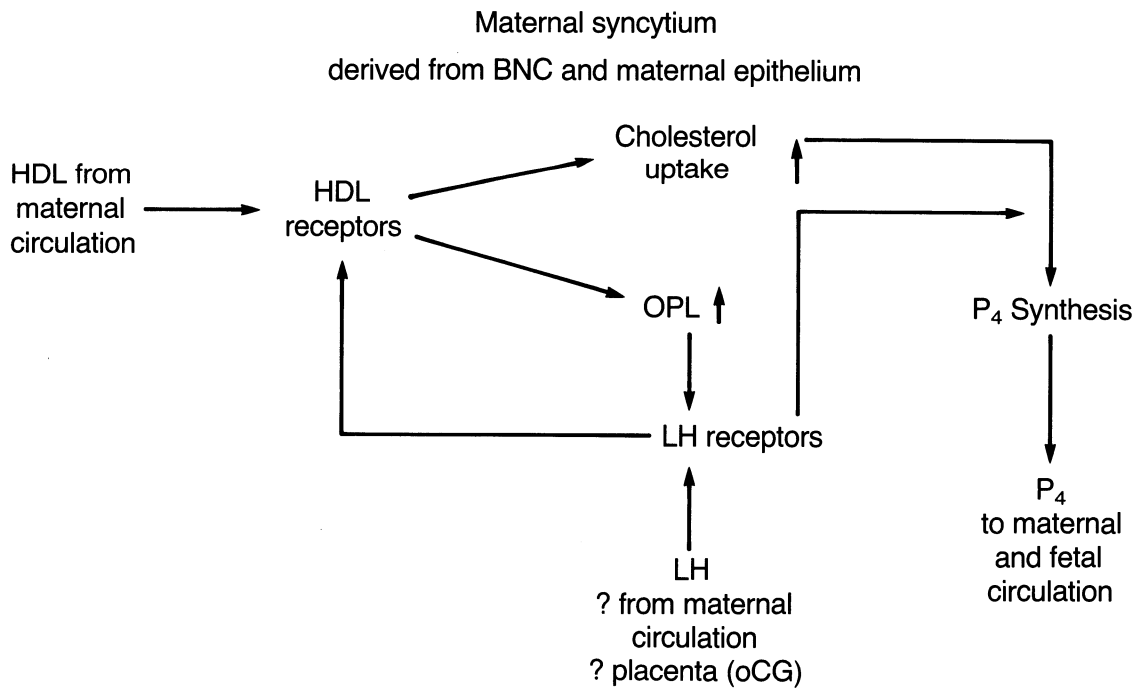

Figure 9.8 The putative mechanisms involved in progesterone biosynthesis in the sheep placenta. The maternal syncytium is thought to be formed by fusion of BNCs derived from the fetal trophoblast and the cells of the maternal epithelium.

species as compared to those in which the CL is the main site of P_4 production, is the development of lipoprotein binding sites on the placenta and their ability to derive cholesterol from the maternal circulation as a substrate for P_4 synthesis.

(e) Chorionic gonadotrophin

Using a radioreceptor assay, Lacroix and Martal (1979) detected hCG-like activity in the ovine placenta and amniotic fluid but not in maternal plasma. This substance first appeared in the placenta as early as day 15, reaching a peak concentration at day 80, and remained constant until near term, when it decreased rapidly (Lacroix and Martal, 1979). Further work is required to establish the presence of CG in the ovine placenta and to determine whether it plays a significant role in placental P_4 production. The significance of large quantities of a GnRH-like material in the ovine placenta (Rice and Smirnis, 1990) has yet to be established.

(f) Prepartum decrease in concentrations

The concentration of P_4 in the plasma of pregnant ewes decreases in the last few days before parturition (Bassett *et al.*, 1969; Fylling, 1970, Stabenfeldt *et al.*, 1972). Concentrations of P_4 and 20α-dihydroprogesterone (20α-P_4) in fetal and maternal plasma fall with increasing gestational age, the mean time of onset of the fall for maternal P_4 being 3.5 ± 0.5 days and for maternal 20α-P 3.4 ± 0.3 days before delivery (Elsner *et al.*, 1980; Magyar *et al.*, 1981; Nathanielsz *et al.*, 1982; Yu *et al.*, 1983). Consistent with earlier reports (Nancarrow and Seamark, 1968; Strott *et al*, 1974) maternal concentrations of P_4 were 10-fold higher than fetal concentrations. Mean fetal 20α-P_4 concentrations were two-/fourfold higher than maternal concentrations. The time courses of the decreases in 20α-P_4 and P_4 were similar (Elsner *et al.*, 1980). An active 20α-reductase exists in the placenta and fetal erythrocytes (Nancarrow and Seamark, 1968), and therefore these results indicate that P_4 is metabolized in the placenta to 20α-P_4 and is subsequently secreted into the fetal and maternal circulations with P_4. Some of the P_4 secreted into the fetal circulation is rapidly converted to 20α-P_4 by the 20α-reductase in the fetal erythrocytes.

The metabolic clearance rate (MCR) of P_4 in blood is high (3.5 l/min) and rises slightly towards the end of pregnancy (Bedford *et al.*, 1972a). The conversion ratio (Longcope *et al.*, 1968) of P_4 to 20α-P_4 is 77.2%. When P_4 is administered in large amounts to pregnant ewes to block parturition, it is rapidly converted to 20α-P_4 and virtually none is converted to oestrogen (Nathanielsz *et al.*, 1988). This is consistent with the observation that the ovine placental pathway is ineffective (Mason *et al.* 1989) and that C_{19} steroids, which become the aromatizable substrates for oestrogen formation, are produced principally from P_5 via 17α-hydroxypregnenolone (17α-P_5) and dehydroepiandrosterone (DHEA), the so-called Δ_5-pathway, rather than 17α-hydroxyprogesterone, which is inefficiently converted to androstenedione (Mason *et al.*, 1989). This would explain why the exogenously added P_4 was not a substrate for ovine placental oestrogen production but was rapidly metabolized to 20α-P_4 and other metabolites (Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988). Mason *et al.* (1989) have suggested that, contrary to the general opinion, the preparturient decline in P_4 concentrations is due to diversion of P_5 metabolism away from 3β-HSD action to 17α-P_5 and DHEA formation.

Pregnenolone therefore emerges as a key steroid in the ovine placenta. Elsner *et al.* (1980) have emphasized the high levels of P_5 and its sulphoconjugate in maternal and fetal plasma. The relative concentrations (ng/ml) of these steroids in maternal and fetal (in parentheses) plasma at approximately 125 days' gestation are as follows: P_5-S 169 (200);

P_5 2.5(5.0); P_4 18.0 (1.5); 20α-P_4 9 (20.0) (Elsner *et al.*, 1980; Nathanielsz *et al.*, 1982). Clearly the ovine placenta secretes considerable amounts of P_5 , which is metabolized to the sulphoconjugate by the sulphotransferase (Rosenfeld *et al.*, 1980) in the placenta and maternal and fetal liver. Nathanielsz *et al.* (1982) suggested that because P_5-S levels decreased before parturition the high concentrations of P_5 -S in the maternal compartment may contribute to the preparturient rise in oestrogen. However, Mason *et al.* (1989) have shown that steroid sulphatase activity is undetectable in the ovine placenta and that sulphated steroids, such as P_5-S, are not substrates for steroid 17α-hydroxylase/C-17,20-lyase. The decreases in P_5-S concentrations observed in maternal and fetal plasma (Nathanielsz *et al.* 1982) are therefore probably due to the metabolism of P_5 into 17α-P_5 and DHEA rather than its sulphation (Mason *et al.*, 1989).

At day 125 of gestation the high circulating concentrations of P_4, 20α-P_4, P_5 and particularly P_4-$_5$S are due to the marked increase in P_5 production from cholesterol between 90 and 125 days. The large amounts of Δ_5 steroids secreted by the placenta indicated that 3β-HSD activity is rate limiting to some extent. However, the profound changes in placental steroid metabolism observed in the last 2–5 days before delivery, resulting in a dramatic decrease in maternal P_4 concentrations, are believed to be a consequence of the prepartum rise in the plasma concentration of cortisol in the fetal circulation (Bassett and Thorburn, 1969; Magyar *et al.*, 1980). Increases in the activities of steroid 17α-hydroxylase (A.B.M. Anderson *et al.*, 1975; N.G. Anderson *et al.*, 1978a) and steroid C-17, 20 lyase (Steele *et al.*, 1976; N.G. Anderson *et al.*, 1978b) are involved.

Between 118 and 140 days of gestation, P_5 and P_4 17α-hydroxylase activities in placental microsomes were undetectable (France *et al.*, 1988; Mason *et al.*, 1989). Only minor metabolism of carbon-labelled P_5 or P_4 substrate occurred with these preparations under the described assay conditions (Trilostane, a 3β-HSD inhibitor, was present). The radiolabelled products did not exceed 4% of the total radioactivity and appeared to be predominantly 20α-reduced products. In these *in vitro* conditions, precise quantification of the low basal activity of steroid 17α-hydroxylase was impeded by the presence of 20α-HSD activity, but this may not occur *in vivo*. A low level of 17α-hydroxylase activity may account for the low level of oestrogen production by the ovine placenta at this stage of gestation. In normal and glucocorticoid-induced parturition there was a marked increase in 17α-hydroxylase activity and it was associated with a marked accumulation of cytochrome $P450_{17\alpha}$ protein. The mechanism of glucocorticoid action in natural ovine parturition may therefore be to increase cytochrome $P450_{17\alpha}$ synthesis either by stabilizing the mRNA for cytochrome $P450_{17\alpha}$ or by acting as a stimulus to transcription of the steroid 17α-hydroxylase gene. Much higher levels of 17α-hydroxylase activity were present in placentae from ewes in which labour was induced with dexamethasone than in those in normal labour, and the level of activity increased with the time in labour. Mason *et al.* (1989) concluded that the differences in activity were due to the dosage and duration of exposure of the placenta to glucocorticoid, whether endogenous or exogenous.

This suggestion is consistent with results of other studies in which premature parturition, secondary to a fetal infusion of cortisol, was blocked by medroxyprogesterone acetate or exogenous P_4 (Thorburn, 1983; Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988) and maternal and fetal oestrone sulphate concentrations continued to increase to high levels, indicating a progressive increase in 17α-hydroxylase activity. Based on the data of Magyar *et al.* (1980), a fetal cortisol concentration of about 20–30 ng/ml over 2–5 days would be needed to induce the necessary

17α-hydroxylase activity and then delivery. Presumably the 17α-hyroxylase activity would continue to increase with the increasing fetal cortisol levels. Mason *et al.* (1989) provided direct evidence that the preparturient decrease in P_4 concentrations is caused not only by metabolism of P_4 to 17α-OH-P_4 and-pregnen-17α,20α-diol-3-one but primarily by diversion of P_5 metabolism from 3β-HSD action to 17α-OH-P_5 and DHEA formation. The substrate specificity of the ovine placental steroid 17α-hydroxylase/C-17,20-lyase is similar to that of bovine and human $P450_{17\alpha}$ and uses P_5, P_4 and 17α-OH-P_5 as substrates, but inefficiently converts 17α-OH-P_4 to androstenedione.

One of the most compelling arguments against P_4 withdrawal being a prerequisite for the initiation of parturition in sheep was the failure of exogenous P_4 administered in physiological amounts to block labour (Liggins, 1973). Bengtsson and Schofield (1963) reported that P_4 (80 mg/day) did not delay the onset of labour at term. Liggins *et al.* (1972) showed that P_4 (100 mg/day for 4 days), in amounts which restored plasma P_4 levels, failed to delay the onset or the progress of labour, a result consistent with earlier observations (Hindson *et al.*, 1969). When the amount of P_4 was increased to 150 mg/day, cervical dilatation was blocked, and only when larger doses of P_4 (e.g. 200 mg/day) were administered was uterine activity blocked and normal or induced delivery period prevented. This amount of P_4 is more than twice the normal production rate for late pregnancy (Bedford *et al.*, 1972a).

When dexamethasone (or cortisol) is administered, the placental 17α-hydroxylase is markedly increased and endogenous P_5 metabolism is diverted away from P_4 formation towards oestrogen synthesis. Exogenous P_4, instead of supplementing endogenous placental P_4, represents the sole source of P_4 in the maternal circulation and is in turn rapidly metabolized to 20α-P_4 and other metabolites (Thorburn, 1983; Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988). Moreover, the metabolism of the endogenous P_5 to oestrogens leads to high plasma oestrogen concentrations. It is only when the exogenous P_4 is increased to 200 mg/day that the blocking activity of P_4 on the myometrium is successful in the face of high oestrogen concentrations. Consistent with this view was the finding that the expected release of $PGF_{2\alpha}$ into the utero-ovarian vein in response to diethylstilboestrol administration was blocked by the same dose of P_4 (200 mg/day). Clearly the release of $PGF_{2\alpha}$ (and hence uterine activity) from the placenta is encouraged by an increase in the oestrogen–P_4 (E/P_4) ratio. The administration of glucocorticoids dramatically increases the E/P_4 ratio, by increasing 17α-hydroxylase activity, and leads to $PGF_{2\alpha}$ release, uterine activity and labour. Large amounts of exogenous P_4 are needed to return the E/P_4 ratio to preparturient levels because the endogenous P_5 is being converted to oestrogen. The mechanism by which P_4 inhibits $PGF_{2\alpha}$ release is unknown. In experiments wherein P_4 withdrawal was induced by inhibitors of 3α-HSD (Taylor *et al.*, 1982; Jenkin and Thorburn, 1985; Ledger *et al.*, 1985), which converts P_5 into P_4, decrease in plasma P_4 concentration for 6 h was sufficient to provoke an increase in the metabolite of $PGF_{2\alpha}$ in the maternal circulation and premature delivery in a majority of animals. Again, there was a relative increase in the E/P ratio and an increase in $PGF_{2\alpha}$ release and uterine activity.

(g) Metabolism of progesterone and pregnenolone

The half-time clearance of radioactive P_4 in pregnant sheep is 10 min and the MCR about 3.5 l/min (Tsang and Hackett, 1979), within the range previously reported (Bedford *et al.*, 1972a). Tsang and Hackett (1979) found that 20α-P_4 accounted for only 10% of the total radioactive metabolites and that the major mechanism of P_4 metabolism is by 5α-

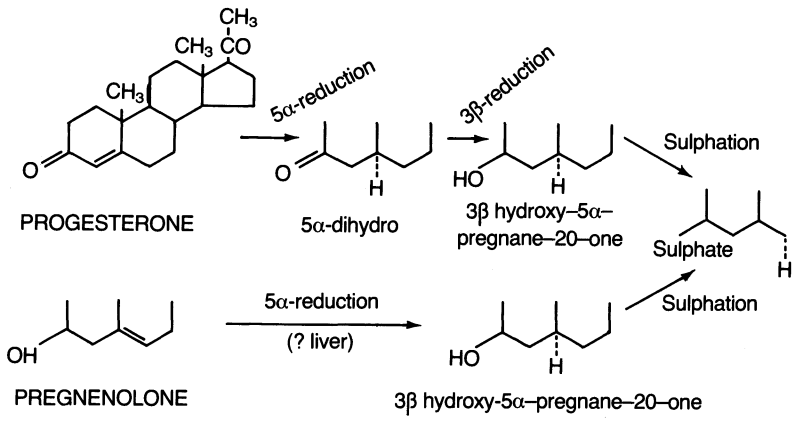

Figure 9.9 Metabolic pathways of progesterone and pregnenolone.

reduction of ring A followed by sulphoconjugation at the 3 position (Figure 9.9).

There appears to be a rapid conversion of the free metabolites to sulphates, which are then slowly cleared from the circulation, possibly due to protein binding, as has been suggested for other steroid sulphates (Wang *et al.*, 1967). The bulk of the metabolites was found in the sulphate fraction, the major one being 3β-hydroxy-5α-pregnan-20-one. While the conversion of P_4 into 20α-P_4 was confirmed, its further metabolism to pregnanediol (5β-pregnane-3α,20α-diol), the prominent human and goat (Schulster *et al.*, 1976) pregnancy metabolite, was not evident either as the free steroid or as the sulphoconjugate (see Figure 9.9). However, small amounts of 5α-pregnane-3β, 20α-diol were found in the sulphate fraction. Although the metabolism of P_5 has not been studied in the sheep, it seems likely that P_5 is sulphoconjugated directly, leading to the high levels of P_5-S measured in the maternal and fetal circulation (Nathanielsz *et al.*, 1982). Some P_5 may undergo initial reduction to its 5α-metabolite, 3β-hydroxy-5α-pregnan-20-one, and then sulphoconjugation. The 5α-reduction is thought to take place in the liver, whereas sulphoconjugation can take place either in the placenta or the liver.

Despite one of their animals being in labour and the other just 2 days before delivery, when it might be expected that placental 17α-hydroxylase activity would be increased, Tsang and Hackett (1979) found no conversion of the labelled P_4 to oestrogens and considered that this may have been due to a failure of P_4 to cross the placenta. Thorburn (1983) and Nathanielsz *et al.* (1988) also found that P_4, when given to block labour, was not converted to oestrogens. However, Mason *et al.* (1989) reported that 17α-OH-P_4 is an unsatisfactory substrate for ovine placental C-17,20-lyase and it will be necessary to inject labelled P_5 to parturient sheep and determine the efficiency of P_5 to oestrogen to clarify this anomaly. It seems unlikely that P_4 failed to 'cross' the placenta (or, more accurately, gain access to the key enzymes) since DHEA when given to the mother is rapidly converted to oestrogens (Rosenfeld and Worley, 1978). The studies of Jenkin *et al.* (1985a,b) indicate that neither P_4 nor 20α-P_4 is transferred from the ewe to the fetus to any great extent.

It has been suggested (Tsang and Hackett, 1979) that 5α-reduction of P_4 may have physiological implications and that this may be related to its biological activity in target organs (Nuti and Karavolas, 1977): 5α-

pregnane-3,20-dione is retained by the ovine endometrium after P_4 infusion (Bedford *et al.*, 1974) and there is a considerable amount of the sulphoconjugate of 5α-pregnan-3β-ol-20-one in sheep pregnancy plasma (Tsang and Hackett, 1979). The biological activity of these steroids needs to be assessed, particularly in relation to the regulation of PG synthesis.

In contrast to the pregnant ewe, 5β-pregnanediols are the major metabolites of P_4 in the fetus. P_4 is produced by the fetal adrenal glands as well as the placenta (Nancarrow, 1969; Cox, 1975; Dolling and Seamark, 1979). The amount of P_4 produced by the ovine placenta near term is about 40–50 mg/day (Bedford *et al.*, 1972a), although the amount entering the circulation is unknown. The three major metabolites of P_4 found in fetal sheep blood are 5β-pregnane-3β,20β-diol, 5β-pregnane-3β, 20α-diol and 5β-3α,20α-diol, all were present as sulphates in μg/ml amounts (Dolling and Seamark, 1979). *In vivo* studies with radioactive tracers have shown these three 5β-pregnanediols to be end products of P_4 metabolism (Nancarrow and Seamark, 1968; Nancarrow, 1969). The enzymes involved include two specific oxidoreductases which catalyse the reversible reduction of the 3-oxo and 20-oxo groups, a 4-ene-5β reductase which catalyses the reversible reduction of the A ring of P_4, and the specific alkyl sulpha-transferases in the conjugate formation. These enzymes are widely distributed in both fetal and placental tissues including blood (Nancarrow and Seamark, 1968; Seamark *et al.*, 1970: Cox, 1975; Dolling and Seamark, 1979). The blood of fetal sheep possesses a remarkable ability to metabolize P_4; the main enzymes include 20α-hydroxysteroid dehydrogenase and 3α-reductase (Seamark *et al.*, 1970). The placenta and other fetal tissues are potent sources of steroid sulphatase activity (Ainsworth, 1972). In control fetuses, the plasma concentrations of both 5β-pregnane-3β, 20β-diol and 5β-pregnane-3β, 20α-diol showed a steady four-fold increase between 105 and 143 days' gestation; a more marked increase was observed in fetuses which came into premature labour following infection or bilateral nephrectomy (Dolling and Seamark, 1979). In this study, the concentrations of these 3β-hydroxy metabolites were higher than those of 5β-pregnane-3α,20α-diol. These results suggest the fetal adrenal glands may be a major source of P_4 in the fetal lamb and that P_4 derived from the adrenal glands is mainly metabolized to these 3β-hydroxy, 5β-pregnane derivatives, whereas P_4 derived from the placenta is metabolized to 5β-pregnane-3β, 20α-diol (5β-pregnanediol). *In vitro* studies with fetal adrenal tissues (A.B.M. Anderson *et al.*, 1970; Glickman *et al.*, 1979) have shown that trophic stimulation of the fetal adrenal before about 130 days' gestation causes an increase in the synthesis of progestagen rather than cortisol, the capacity to synthesize cortisol developing after this time (see Dolling and Seamark, 1979). With the rapid increase in cortisol concentration in the four to five days before labour, there is an associated decrease in fetal concentration of the 3β-hydroxy, 5β-pregnanes (Dolling and Seamark, 1979), suggesting that 17α-hydroxylase activity has increase in the fetal adrenal cortex. These studies confirm that the fetal adrenal glands are producing considerable amounts of P_4 (as well as corticosterone (Madill and Bassett, 1973) and possibly some pregnenolone) for some time before it commences secreting cortisol. It would appear that using plasma cortisol concentration as the sole criterion of fetal adrenal steroidogenic activity may be misleading.

A similar situation exists in the fetal foal, where it has been shown that the fetal adrenal glands secrete pregnenolone (and possibly P_4) for some weeks before delivery and only in the last one to two days do the plasma cortisol levels increase (Thorburn, 1993). Administration of synthetic ACTH to immature fetal foals causes an increase in the maternal and fetal plasma levels of 5β-pregnane

metabolites of pregnenolone and P_4 (see Thorburn, 1993).

9.5.3 OESTROGENS

In 1958, Velle reported that urine from late pregnant sheep contained significant quantities of oestradiol-17α and oestrone. Fèvre and Rombauts (1966) confirmed that these were the major oestrogens excreted during pregnancy and found that oestrogens first appeared in the urine at about day 70 and their excretion increased significantly from about day 90 to term. Ovariectomy after day 60 did not markedly affect the pattern of oestradiol-17α excretion, although oestrone excretion was reduced (Fèvre, 1967), indicating that these oestrogens are mainly derived from the fetoplacental unit. Until day 120, oestrogen sulphoconjugates in fetal plasma (largely oestrone and oestradiol-17α) increased in a similar pattern but then decreased (Findlay and Cox, 1970; Findlay and Seamark, 1973; Carnegie and Robertson, 1978) before undergoing a massive increase in the last 2–4 days before delivery (Currie *et al.*, 1973; Nathanielsz *et al.*, 1982).

The concentration of oestrogen sulphoconjugates in maternal plasma provides a better index of placental oestrogen biosynthesis. Carnegie and Robertson (1978) described three distinct stages of oestrogen biosynthesis by the ovine placenta. The first stage covers the period day 0–55, the second day 55–140 and the third the last 2–4 days of gestation. The major oestrogens present in maternal plasma are the sulphoconjugates of oestrone and oestradiol-17α and their concentrations increase progressively from day 70 to term (Currie *et al.*, 1973; Carnegie and Robertson, 1978). Nathanielsz *et al.* (1982), in a detailed study of the preparturient hormone profiles, observed a fall in fetal and maternal oestrone sulphate concentrations from 10 to 5 days before birth followed by a terminal rise over the last 24–48 h that was a mirror image of the fall in plasma P_5, P_5-S and P_4 values. The preponderance of sulphotransferase over sulphatase activity in the fetoplacental tissues of the sheep results in rapid sulphoconjugation of oestrogens (Currie *et al.*, 1973; Findlay and Seamark, 1973; Thorburn *et al.*, 1977a) and accounts for the predominance of oestrogen sulphoconjugates over unconjugated oestrogens in fetal and maternal plasma.

The maternal plasma concentrations of unconjugated oestrogens (oestrone and oestradiol-17β) are low until day 120 then increase gradually before undergoing a sudden and rapid rise in the 24 h before parturition (Challis, 1971; Challis *et al.*, 1971; Bedford *et al.*, 1972b; Thorburn *et al.*, 1972a; Robertson and Smeaton, 1973; Challis and Patrick, 1981). This dramatic increase in free oestrogens coincides with the major increase in the oestrogen sulphoconjugates in fetal and maternal plasma at that time. Since the oestrogen MCR did not change (Challis *et al.*, 1973a), the preparturient increase in free oestrogen concentrations may simply be an expression of a massive increase in the production rate of oestrogen by the ovine placenta in the 24–48 h before delivery, although an increase in placental sulphatase or aromatase activities cannot be excluded.

The prepartum rise in fetal plasma concentrations of cortisol increases the activity of steroid 17α-hydroxylase (A.B.M. Anderson *et al.*, 1975; N.G. Anderson *et al.*, 1978a; France *et al.*, 1988; Mason *et al.*, 1989) and steroid C-17,20-lyase (Steele *et al.*, 1976). In microsomes of placental cotyledons obtained from pregnant ewes at 118–140 days' gestation, in which there was no evidence of active or impending labour, steroid 17α-hydroxylase activity was below the sensitivity of the assay (France *et al.*, 1988), but administration of dexamethasone to the fetus for 45 h dramatically increases the amounts of 17α-hydroxylase. The activity of 17α-hydroxylase is increased in placenta from ewes in labour (Mason *et al.*, 1989), but dexamethasone is a more potent stimulus for increasing 17α-

hydroxylase activity than are the cortisol concentrations in normal labour. The increased 17α-hydroxylase activity in dexamethasone-induced parturition presumably explains the higher circulating oestrogen values in this type of premature labour. The 17α-hydroxylase activity is determined not only by the dosage but also by the duration of exposure of the placenta to the glucocorticoid (France *et al.*, 1988; Mason *et al.*, 1989). If parturition is blocked by the maternal administration of medroxyprogesterone acetate or P_4 there is almost a complete diversion of P_5 metabolism through to oestrogens, and P_4 and 20α-P_4 virtually disappear from the fetal and maternal circulations and oestrogen concentrations (particularly oestrone sulphate) reach very high levels (Thorburn, 1983; Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988). In these studies the fetal infusion of cortisol was maintained for several days and the oestrogen concentrations progressively increased with time.

Therefore, like the plasma oestrogen concentrations, 17α-hydroxylase activity only reaches measurable levels in the last 2–4 days before delivery, whereas P_4 and P_5 concentrations decrease over the same period. Fetal cortisol concentrations must therefore reach high levels to induce sufficient 17α-hydroxylase activity to cause a decrease in P_4 and an increase in oestrogen levels. Dexamethasone, apart from being a potent glucocorticoid, is not bound to protein whereas, at term, about 85–90% of the circulating cortisol is bound to protein (Ballard *et al.*, 1982). Therefore, only about 6 ng/ml 'free' cortisol is needed in the fetal circulation to induce sufficient 17α-hydroxylase activity to induce parturition. The gradual increase in fetal cortisol concentrations before the last 4–5 days, i.e. up to day 140 of gestation, may play a key role in the maturation of a number of organ systems, including the fetal pituitary and adrenal.

It has been generally accepted that oestrogen synthesis in late gestation is largely an intraplacental function and that sheep, unlike primates, do not have a fetoplacental unit for oestrogen biosynthesis (B.F. Mitchell *et al.*, 1986). However, these experiments have not excluded a role for C_{19} steroids of fetal adrenal origin in oestrogen synthesis (Liggins *et al.*, 1972, 1973; Thorburn *et al.*, 1977a; Thorburn and Challis, 1979; Challis and Olson, 1988). The concentrations of androstenedione and testosterone in fetal sheep plasma rise before parturition (Findlay and Seamark, 1973; Yu *et al.*, 1983), and it is possible that circulating androgen precursors could contribute to the increasing oestrogen production. B.F. Mitchell *et al.* (1986) showed that androstenedione is cleared rapidly from the fetal circulation and that it has an extremely high MCR. In these studies, pulsatile ACTH administration to the fetus did not alter the high clearance rate of androstenedione but caused significant increases in its production rate and whole blood concentration. The production rate for androstenedione was significantly increased even when the rise in fetal cortisol had effectively been blocked by concomitant administration of metyrapone, an 11β-hydroxylase inhibitor. This experiment suggests that the increase in production rate of androstenedione was due to activation of the fetal adrenal. Furthermore, when tritiated androstenedione was infused into chronically catheterized fetal sheep, radioactive oestrogen conjugates were isolated and identified from fetal and maternal circulations (B.F. Mitchell *et al.*, 1986). It was estimated that up to 30% of maternal oestrogens at term might be formed in the placenta from fetal adrenal precursors (Challis and Olson, 1988). Collagenase-dispersed fetal adrenal cells secrete androstenedione, (B.F. Mitchell *et al.*, 1986) and the output by these cells, when treated with $ACTH_{1-23}$ *in vitro*, was significantly greater when the tissue was obtained from animals that had previously received an ACTH infusion *in vivo* than from saline-treated controls. Currie *et al.* (1973) had

shown that the infusion of androstenedione into a fetal lamb provoked an increase in maternal unconjugated oestrogen levels and precipitated premature parturition, although DHEAS infused into the fetus in similar amounts was ineffective. In such experiments high levels of oestrogen need to be produced to induce parturition because placental P_4 production would be maintained. Further experiments are needed to confirm these observations.

A bolus injection of DHEA (10 mg) into the mother resulted in an increase in unconjugated oestrogen in both the fetal and maternal compartments (Pupkin *et al.*, 1975). Rosenfeld *et al.* (1977) similarly showed that an infusion of DHEA into the fetus resulted in the placental production of oestrogen.

In summary, it seems likely that both the adrenal and the placenta provide C_{19} steroids as precursors for placental aromatization and that both contribute to the preparturient oestrogen surge.

9.5.4 AROMATASE

Ovine placental aromatase is principally located in the microsomes (Ainsworth and Ryan, 1966), however France *et al.* (1987) concluded that, although the endoplasmic reticulum of the ovine placental cells is a major site of aromatase, other membrane structures in the cell also appear to contain the aromatase enzyme system and may be of more importance overall to a total aromatase activity. Sheep placental microsomes metabolize C_{19} steroids to oestrone and oestradiol *in vitro* (Ainsworth and Ryan, 1966; Pierrepoint *et al.*, 1971; Findlay and Seamark, 1973; Mann *et al.*, 1975; France *et al.*, 1987, 1988; Mason *et al.*, 1989). Basal levels of placental aromatase are relatively constant between 118 and 140 days' gestation and a two- to threefold increased in the placentas of ewes in normal or dexamethasone-induced labour (Mann *et al.*, 1975; Mason *et al.*, 1989). Aromatase activity increases in placental explants when cortisol is added to the culture medium (Ricketts *et al.*, 1980; Flint, 1983), and glucocorticoids in high concentration may induce a modest increase in aromatase activity. Mason *et al.* (1989) questioned the significance of the preparturient increase in aromatase activity, because earlier studies (Rosenfeld *et al.*, 1980; France *et al.*, 1987) suggested that aromatase did not appear to be a rate-limiting enzyme with regard to oestrogen production; the basal levels of the enzyme found between 118 and 140 days' gestation appeared adequate to accommodate the rise in oestrogen production which occurs in the last 48 h of pregnancy.

Information is lacking on the level of aromatase activity in the placenta of the sheep during the critical phase between days 70 and 120 when placental oestrogen biosynthesis is increasing and there is a steady increase in the concentration of oestrone sulphate in fetal and maternal plasma. Thorburn *et al.* (1989) have suggested that fetal pituitary FSH may regulate placental aromatase because fetal hypophysectomy at day 70–75 prevented the increase in maternal oestrone sulphate levels that normally occurs between 90 and 120 days' gestation. Kendall *et al.* (1977) found that, when parturition was induced by administering ACTH to hypophysectomized fetuses, there was no increase in maternal unconjugated oestrogen concentrations, although there was a fall in P_4 and a rise in PG output. They suggested that some pituitary factor may be required for placental oestrogen biosynthesis, and the results of Thorburn *et al.* (1989) would be consistent with the proposal that FSH may be the fetal pituitary factor.

The discussion so far has centred around the rapid increase in oestrogen concentrations in the last 2–4 days before delivery (Currie *et al.*, 1973; Nathanielsz *et al.*, 1982), but little attention has been directed towards the gradual increase in placental oestrogen production from day 70 to day 120–130 (Fèvre and Rombauts, 1966; Currie *et al.*, 1973;

Carnegie and Robertson, 1978). Although the placental 17α-hydroxylase activity is low between 118 and 140 days' gestation (France *et al.*, 1988), it is possible that sufficient P_5 is metabolized by the low levels of 17α-hydroxylase to explain the progressive increase in oestrogen production. It is also possible that C_{19} steroids, derived from the fetal or the maternal adrenal, may act as substrates for oestrogen biosynthesis. However, the pattern of increase of maternal plasma oestrone sulphate levels so closely follows that of P_4 it would favour the former suggestion that there is a leak in the placental steroidogenic pathway and that some P_5 is diverted through to oestrogens.

The progressive increase in the oestrone sulphate concentrations in the maternal plasma is of particular importance to the development of the myometrium. Oestradiol-17β stimulates synthesis of the contractile protein, actomyosin, and lowers the threshold of stimulation of the myometrial cell to oxytocin. Sulphatase, sulphotransferase and 17α- and 17β-HSD are present in sheep myometrium, and oestradiol-17β is the major product formed from oestrone and oestrone sulphase (Fitzpatrick, 1960; Kuriyana, 1961; Knifton, 1967; Brinsfield, 1968; Rossier and Pierrepoint, 1974). In addition, oestrone sulphate is converted to oestrone and oestradiol by ovine endometrium (Dwyer and Robertson, 1980; Power and Challis, 1987). The concentrations of oestrogens (pg/g) in myometrium and endometrium exceed those in plasma (pg/l) (Challis and Patrick, 1981), indicating that enzymatic interconversions and tissue concentrating mechanisms, including specific receptor uptake, affect tissue oestrogen concentrations with the possibility of producing local changes that may not be apparent in plasma measurements (Power and Challis, 1987). The concentration of oestrone and oestradiol in myometrium increases between day 130–135 and term in animals in spontaneous labour or after ACTH-induced labour (Power and Challis, 1987). Oestrogens may promote gap junction formation between myometrial cells (Garfield *et al.*, 1987) and stimulate the formation of oxytocin receptors (Alexandrova and Soloff, 1980a–c) and the output of stimulatory PGs (Liggins *et al.*, 1973) in the uterus. These observations confirm and extend the previous measurements of total oestrogen by Rawlings and Ward (1976). Power and Challis (1987) showed that the concentrations of oestrone and oestradiol in the fetal membranes were higher than in the endometrium and myometrium. In the amnion there was no consistent change in oestrone concentrations with gestation, although oestradiol concentrations increased between day 130 and term. Concentrations of oestrogen in the chorion after day 100 were the highest of all tissues, but did not change between day 130–135 and term (Power and Challis, 1987), although the chorion is a major site of PG production and metabolism (C.A. Evans *et al.*, 1982). Oestrone sulphatase is present in the sheep chorion and the chorion is also able to aromatize C_{19} steroids; local enzymatic regulation of chorionic oestrogens may therefore be important in the control mechanisms of pregnancy and parturition (Power and Challis, 1987).

9.5.5 PROSTAGLANDINS

(a) Plasma concentrations of $PGF_{2\alpha}$ and PGFM

Normal parturition in sheep is associated with a marked increased in concentrations of $PGF_{2\alpha}$ in uterine venous plasma (Liggins and Grieves, 1971; Thorburn *et al.*, 1972a). Concentrations of PGE_2, PGFM (the major metabolic of $PGF_{2\alpha}$) and 6-keto-$PGF_{1\alpha}$ (the major metabolite of prostacyclin, PGI_2) are also increased before spontaneous labour (M.D. Mitchell *et al.*, 1979a; C.A. Evans *et al.*, 1981) and before labour induced by intrafetal infusion of ACTH or glucocorticoids (Currie *et al.*, 1973; Thorburn *et al.*, 1977b; M.D.

Mitchell *et al.*, 1979a). Similarly, the concentrations of PGE_2 in fetal plasma (Challis *et al.*, 1976a) and of PGE_2 , $PGF_{2\alpha}$ and 6-keto-PGF_1 α in amniotic fluid (Challis *et al.*, 1978; M.D. Mitchell *et al.*, 1978e; Olson *et al.*, 1985) increase before delivery.

Detailed information on the plasma concentrations of $PGF_{2\alpha}$ and PGFM throughout pregnancy is lacking. PGFM concentrations in the utero-ovarian vein accurately reflect those of $PGF_{2\alpha}$ (Burgess *et al.*, 1989), and Burgess (1991) showed that basal concentrations of PGFM in utero-ovarian vein plasma are low (0.36 ± 0.05 nM) on days 60–63 of gestation but then gradually increase to 1.78 ± 0.28 nM by days 135–138, and 3.48 ± 0.35 nM in preparturient ewes (140–147 days) before reaching high levels at parturition (19.2 ± 2.5 nM). The arterial concentrations of PGFM were much lower than those in the utero-ovarian vein; at days 60–63 PGFM was undetectable in arterial plasma, and by days 135–138 its concentration had only reached 0.82 ± 0.07 nM. The arteriovenous differences showed that PGFM is secreted by the uterus in concentrations equimolar to those of $PGF_{2\alpha}$. The production of $PGF_{2\alpha}$, as judged from PGFM values, was low at mid-gestation, before increasing gradually during the late gestation and then dramatically in the last 24 h before delivery.

The arteriovenous difference for PGFM across the uterus confirms the earlier studies of M.D. Mitchell *et al.* (1980c) and Fowden and Silver (1983) and demonstrates that the ovine uterus metabolizes some of the $PGF_{2\alpha}$ it produces *in vivo* and secretes it as its metabolite PGFM. PGFM appearing in the uterine vein is therefore derived from $PGF_{2\alpha}$ delivered to the uterus in its arterial circulation plus PGFM synthesized in the uterine tissues (Keirse *et al.*, 1976; M.D. Mitchell and Flint, 1978a).

In the pregnant uterus, 83% of the uterine blood flow is distributed to the cotyledons, 3–4% to the myometrium and 13–14% to the endometrium (Makowski *et al.*, 1968). Since the $PGF_{2\alpha}$ production rate by the myometrium *in vitro* is only about 25% of that of the maternal cotyledon (M.D. Mitchell and Flint, 1977), the maternal cotyledons appear to be the major contributor to $PGF_{2\alpha}$ concentrations in the utero-ovarian vein (Thorburn and Challis, 1979).

It has been assumed that most of the PGF produced by the uterine tissues will be metabolized in the lungs (Vane, 1978) and that little, if any, would appear in the arterial circulation. However, Fowden *et al.* (1987) and Andrianakis *et al.* (1989a) have shown that some PGE_2 escapes metabolism in the lungs and appears in significant amounts in the arterial circulation. Small amounts of $PGF_{2\alpha}$ are present in maternal arterial plasma during ACTH-induced preterm labour (Olson *et al.*, 1985). More information is needed on arterial concentrations of $PGF_{2\alpha}$; if $PGF_{2\alpha}$ does not reach the myometrium by a systemic route, it must be produced locally by the membranes and endometrium and reach the myometrium by diffusion (Thorburn and Challis, 1979). Alternatively, the increasing amounts of PGE_2 in the arterial circulation (see below) may exert a direct stimulatory action on the myometrium or be converted locally to $PGF_{2\alpha}$ by 9-keto-reductase.

(b) Prostaglandin E_2

Using a new indirect assay for PGE_2, in which the more stable methyloxime derivative of PGE is formed (R.W. Kelly *et al.*, 1986), Fowden *et al.* (1987) reported that the concentrations of PGE_2 in the maternal carotid artery, uterine vein and the fetal carotid artery increase progressively during the last third of gestation. An arteriovenous difference for PGE_2 on the maternal and fetal sides of the placenta (Fowden *et al.*, 1987; Andrianakis *et al.*, 1989a,b) indicates that the increasing plasma concentrations are an expression of increased PGE_2 production by the placenta.

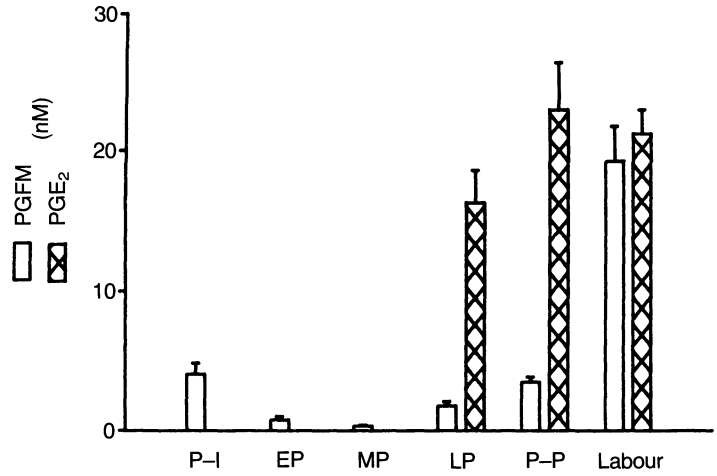

Figure 9.10 Mean (± SEM) basal utero-ovarian (UOV) venous plasma concentrations of PGFM and PGE_2 during preimplantation (P-I; day 14 p.c.), early pregnancy (EP; day 21–23 p.c.), mid-pregnancy (MP; day 60–63 p.c.), late pregnancy (LP; day 135–138 p.c.) and before (P-P; >day 140 p.c.) and during active labour. Concentrations of PGE_2 in UOV plasma of P-I, EP and MP ewes were below the lower limits of sensitivity of the PGE_2 assay. (Reproduced with permission from Burgess *et al.*, 1991.)

The temporal pattern of PGE production by the uterus differs from that of $PGF_{2\alpha}$: concentrations in the utero-ovarian vein are undetectable in mid-gestation (63–65 days), but reach high levels (16.21 ± 0.83 nM) by late gestation (135–138 days) (Burgess, 1991). The basal PGE_2 concentration in preparturient ewes (22.90 ± 3.4 nM >140 days' gestation) and parturient ewes (32.25 ± 1.7 nM) are not significantly greater than those in the late pregnant ewes, as found by Fowden *et al.* (1987) and Andrianakis *et al.* (1989a). The arterial concentration of PGE_2 at 135–138 days gestation was 4.93 ± 0.24 nM (Burgess, 1991) which is in agreement with the earlier studies. The difference in the temporal pattern of placental PGE_2 and $PGF_{2\alpha}$ production (Figure 9.10) and the vastly different response of PGE_2 and $PGF_{2\alpha}$ to an oxytocin challenge suggests different rates of production and modes of regulation.

(c) Sites of production

Concentrations of $PGF_{2\alpha}$ are higher in maternal cotyledons and myometrium than in fetal cotyledons (Liggins and Grieves, 1971), but there are high levels of PGE_2 in fetal cotyledons, but not myometrium or maternal cotyledons, after delivery (M.D. Mitchell and Flint, 1977). Subsequent work has confirmed these early studies. Using a superfusion system, M.D. Mitchell and Flint (1978b) showed that PGE_2 output from the combined fetal and maternal cotyledons exceeded that of $PGF_{2\alpha}$, a finding consistent with the observation of C.A. Evans *et al.* (1981) that the concentration of PGE in whole cotyledons exceeded that of $PGF_{2\alpha}$.

C.A. Evans *et al.* (1981) concluded that the cotyledons and the chorioallantois were the major sites of PG synthesis: in control animals, the chorioallantois contained the highest concentrations of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ but not PGE_2 were elevated by fetal ACTH treatment. C.A. Evans *et al.* (1981) also proposed that the fetal membranes are major sites of PG production in late pregnancy and during induced labour. The chorioallantois is anatomically close to the endometrium and PGs

synthesized in the chorion might be expected to diffuse readily into the amniotic and allantoic fluids and the concentrations of PGs in these fluids might be an expression of PG synthesis by the chorion. Information on the PG concentrations in allantoic fluid is unavailable. The amniotic fluid becomes more viscid during late gestation, making it more difficult to sample, but concentrations of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in amniotic fluid are elevated before delivery (M.D. Mitchell *et al.*, 1977a,b; Challis *et al.*, 1978). During ACTH-induced labour, PGs increase in fetal fluids before the increased mechanical activity of the uterus begins (Olson *et al.*, 1985), providing further evidence that PGs initiate uterine activity rather than result from it.

The placenta has been clearly identified as a major site of PG production, with the maternal placenta being suggested as the major source of $PGF_{2\alpha}$ (Liggins and Grieves, 1971) and the fetal placenta (trophoblast) of PGE_2 production (M.D. Mitchell and Flint, 1977). The synthesis of PGs by the placenta throughout the pregnancy has been investigated in detail.

PG synthesis by dispersed ovine cotyledonary cells, mostly uninucleate trophoblast cells, is low during early and mid-gestation but increases markedly from day 110–120 onwards (Risbridger *et al.*, 1985). The addition of arachidonic acid to incubations of cotyledonary cells did not significantly enhance PG synthesis, suggesting that the concentration of PGHS in the trophoblast varies during gestation, increasing only after 110 days' gestation.

Microsomes from ovine cotyledons represent a PGHS-enriched subcellular fraction (Leach-Harper and Thorburn, 1984; Risbridger *et al.*, 1985; Rice *et al.*, 1987, 1988) and their capacity to synthesize PGE_2 and $PGF_{2\alpha}$ from radiolabelled arachidonic acid increases 25-fold between 20 and 140 days' gestation, the greatest increase being after 120 days (Rice *et al.*, 1988). This was considered to be the result of an increase in PGHS activity or content in the placenta but could have been due to an increase in the activity or content of the E-isomerase (which converts the product of PGHS activity to PGE_2). This possibility was excluded by studies of the amount of oxygen consumed by the PGHS-catalysed conversion of arachidonic acid to PGs (Rice *et al.*, 1990). Microsomal oxygen consumption increased 22-fold between 74 and 147 days' gestation; it was stimulated by the addition of arachidonic acid and inhibited by inhibitors of PGHS activity such as indomethacin and 4-aminoantipyrine. The increase did not occur after fetal hypophysectomy at 70–80 days' gestation (Rice *et al.*, 1990), consistent with the finding that placental PGE_2 production did not increase during late gestation in such pregnancies (Thorburn *et al.*, 1989). It has been suggested that some fetal pituitary factor may be needed for the induction of PGHS activity, mRNA translation or PGHS gene expression (Thorburn *et al.*, 1989; Rice *et al.*, 1990).

The *in vitro* measurements of the gestational changes in PG-forming capacity (Risbridger *et al.*, 1985), cotyledonary PGHS activity (Rice *et al.*, 1988, 1990) and *in vivo* measurements of plasma concentrations of PGE_2 during gestation (Fowden *et al.*, 1987) show a similar profile: low during early to mid-gestation but increasing rapidly from 110–120 days of gestation to term. Studies of the synthesis of PGs by intrauterine tissues of animals of other species (see Rice *et al.*, 1990) are consistent with the observations in sheep and suggest a common governing mechanism. Thorburn *et al.* (1988, 1991) and Thorburn and Rice (1990) propose that the increased PG production by the placenta during the last 0.25–0.30 of gestation corresponds to the rapid growth phase of the fetus, which commences at about 110–120 days' gestation in the sheep. Reducing the food intake of late pregnant ewes increases placental PGE_2 production, (Fowden *et al.*,

1987), and the increase in substrate demand by the fetus on the placenta during its phase of rapid growth might provide a stimulus for the placenta to increase PGE_2 production with this increased PGE_2 concentration in turn facilitating transport of substrate for the increase in growth of the fetus. Thorburn (1991) has also proposed that the increased concentration of PGE_2 in fetal plasma may provide a stimulus for the fetal hypothalamo-pituitary–adrenal axis, and this may be part of the trigger mechanism for parturition.

Since the pattern of growth of a fetus is genetically determined and is common to most species, it may explain why placental PG production follows a similar pattern in the sheep, pig, rabbit, guinea pig and goat. Under various adverse conditions (e.g. fetal and placental hypoxaemia, maternal undernutrition, placental trauma, hyperthermia) placental PGE_2 production is increased and may lead to premature labour. It is not clear at the present time whether the mechanism (placental PGE_2 production) represents the 'trigger' for parturition or an amplifying system in the activation of the HPA axis (see Chapter 10, this volume).

(d) Differential regulation of release of PGE_2 and $PGF_{2\alpha}$

The belief that PGE_2 and $PGF_{2\alpha}$ are not only synthesized in different sites (Liggins and Grieves, 1971; Mitchell and Flint, 1977) but that their release is regulated in different ways is supported by the studies of Burgess *et al.* (1989) and Burgess (1991). Administration of oxytocin to the ewe significantly increased the release of $PGF_{2\alpha}$ into the utero-ovarian vein but failed to stimulate PGE_2 release. Oxytocin, acting via oxytocin receptors and the IP_3 pathway, may therefore increase the production of $PGF_{2\alpha}$ from the maternal placenta. The failure to stimulate PGE_2 release may be due to the inability of oxytocin to cross the maternal syncytium to gain access to the trophoblast (fetal placenta) and/or to a lack of oxytocin receptors on the trophoblast (Jenkin *et al.*, 1989). The release of $PGF_{2\alpha}$ from the maternal placenta therefore appears to be regulated by the prevailing oxytocin concentrations in maternal plasma and the density of the oxytocin receptors in the placenta. The influence that the fetus has on PGF release into the maternal circulation is by varying the concentrations of oestrogens and P_4 in the maternal plasma, an increase in the E/P ratio causing an increase in the number of oxytocin receptors. Administration of mifepristone, a P_4 antagonist, to a pregnant ewe led to increased concentrations of PGE_2 and $PGF_{2\alpha}$, but only $PGF_{2\alpha}$ increased in response to oxytocin (Burgess *et al.*, 1991), again suggesting a differential response.

The influence of steroid hormones on oxytocin receptors is well documented (Soloff, 1975; Hsueh *et al.*, 1976; Roberts *et al.*, 1976; Nissenson *et al.*, 1978). Oestrogen promotes the synthesis of its own receptor and that of oxytocin (Hsueh *et al.*, 1976; Pavlik and Coulson, 1976; Clark *et al.*, 1977), and this action is antagonized by P_4. The release of PGFM into the utero-ovarian vein in response to oxytocin varies in relation to the prevailing E/P ratio, the higher the ratio the greater the release of $PGF_{2\alpha}$ (Burgess, 1991). The prepartum increase in the E/P_4 ratio in the pregnant ewe might therefore be expected to cause an increase in oxytocin receptors in the maternal placenta in a manner similar to that described in the non-pregnant ewe (Hixon and Flint, 1987). Such an increase has been demonstrated indirectly by the increase in the release of PGFM but not PGE_2 in response to oxytocin after treatment with mifepristone in late pregnant ewes (Burgess *et al.*, 1992). This result is more meaningful than measuring oxytocin receptors *per se* because Sheldrick and Flint (1986) demonstrated that oxytocin receptor numbers were increased even when release of $PGF_{2\alpha}$ in response to oxytocin was inhibited, and Vallet *et al.* (1990) have demonstrated a dissociation between oxytocin receptor concentrations

and release of $PGF_{2\alpha}$ in response to oxytocin in the ewe. Oxytocin concentrations in maternal and fetal plasma rise at parturition (Glatz *et al.*, 1981; M.D. Mitchell *et al.*, 1982a; Dawood *et al.*, 1983), the maternal concentrations increasing significantly during the second stage of labour (Glatz *et al.*, 1981). $PGF_{2\alpha}$ release into the maternal circulation is therefore determined by changes in the concentration of oestrogens, P_4, oxytocin and its receptors, whereas the release of PGE_2 is regulated in a different way and is insensitive to the action of oxytocin.

(e) Route of transfer of PGs to myometrium

The cotyledons represent the major site of PG production in the uterine tissue, but it is still not clear to what extent the PGs arising from the cotyledons reach the myometrium and to what extent they contribute to uterine activity. From the anatomy, Thorburn and Challis (1979) considered it unlikely that PGs could diffuse from the cotyledons to the myometrium and a countercurrent mechanism to transport $PGF_{2\alpha}$ selectively from the uterine vein to the uterine artery (and thus to the myometrium) could not be demonstrated (Liggins *et al.*, 1973). The systemic route of transfer of PGs from the cotyledons to the myometrium would appear inefficient in view of the ability of the lung to metabolize and clear most PGF_2 in the pulmonary circulation (Vane, 1978). However, there is still a lack of information on $PGF_{2\alpha}$ concentrations in maternal arterial plasma during late pregnancy and parturition. An intra-aortic infusion of $PGF_{2\alpha}$ (5–10 μg/min) in pregnant sheep, which Thorburn and Challis (1979) calculated should achieve plasma arterial concentrations of 2–4 ng/ml, increased uterine activity and myometrial sensitivity to oxytocin (Liggins *et al.*, 1973) despite high P_4 concentrations. Thorburn and Challis (1979) proposed that during the last 24 h before delivery, when the sensitivity of the myometrium to $PGF_{2\alpha}$ had increased markedly, due to the decrease in P_4 levels (and a removal of the 'P_4 block') and an increase in oestrogen levels, the myometrium may respond to quite low arterial $PGF_{2\alpha}$ concentrations (Thorburn *et al.*, 1977a). PGs in the arterial circulation may also stimulate oxytocin secretion, thereby reinforcing the reflex-stimulated oxytocin release that occurs at this time in response to cervical and vaginal stretching (Roberts and Share, 1968; Gillespie, 1973; Flint *et al.*, 1975).

Concentrations of PGE_2 in arterial plasma increase during the last third of gestation, reaching high levels at term (Fowden *et al.*, 1987; Burgess, 1991), suggesting that the lungs may be less effective at clearing PGE_2 or that the amounts of PGE_2 presented to the lungs are greater. Nevertheless, the myometrium, cervix and other tissues are exposed to increasing concentrations of PGE_2, which may explain the evolution of uterine activity, and the appearance of the so-called 'contractures' (Nathanielsz *et al.*, 1977), during this time. The pattern of prolonged uterine contractions ('contractures') is thought to be due to the myometrium being stimulated by an agonist while the blocking action of P_4 on the myometrium is present (Thorburn *et al.*, 1983). The propagation of electrical activity in the myometrium is probably blocked by P_4. With the preparturient decrease in P_4 concentrations, the uterine sensitivity to PGs increases markedly (Jenkin *et al.*, 1982; Thorburn *et al.*, 1983; Jenkin and Thorburn, 1985; Burgess *et al.*, 1991). It is not known whether PGE_2 would be equally effective in stimulating uterine activity in sheep.

(f) Endometrium and fetal membranes

In sheep the myometrium is lined by the intercotyledonary endometrium to which the chorion is closely apposed. PGs produced in these tissues can readily diffuse into the myometrium. In the non-pregnant ewe, the endometrium represents a major site of PG production and the PGF_2 produced by the

endometrium is responsible for the increased uterine activity at the end of the oestrous cycle. In the late pregnant ewe, the endometrium can also produce PGs, although *in vitro* studies indicate that the synthetic capacity of the endometrium is less than that of the chorioallantois or cotyledons (Mitchell and Flint, 1977, 1978a; C.A. Evans *et al.*, 1981, 1982; Olson *et al.*, 1985). Nevertheless, the proximity of the endometrium to the myometrium would ensure an efficient transfer of PGs so that as the inhibitory influence of P_4 at the myometrium wanes, PGs produced locally may play a key role in stimulating uterine activity.

In the late pregnant sheep the chorion is extensive and on one surface is in close proximity to the endometrium while on the other it is in close apposition to the allantois and amnion. The chorioallantois contained the highest concentrations of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in late pregnant sheep, and $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ but not PGE_2, were increased after infusion of ACTH to the fetus to induce premature labour (C.A. Evans *et al.*, 1981). It seems likely that PGs produced in the endometrium or chorioallantois can diffuse into the myometrium to stimulate activity. PGs injected into the amniotic or allantoic sacs of sheep during mid-pregnancy can induce abortion (Thorburn, 1977). Extra-amniotic infusion of $PGF_{2\alpha}$ causes a rapid elevation of maternal and fetal plasma and amniotic fluid PGFM concentrations in excess of those found at normal term (Jenkin *et al.*, 1982). When a 3β-HSD inhibitor was injected during extra-amniotic infusion of $PGF_{2\alpha}$ there was a precipitous decline in maternal plasma P_4 concentrations and parturition began within 24 h. They concluded that a substantial decrease in plasma P_4 concentrations is necessary to render the myometrium sensitive to $PGF_{2\alpha}$ in late pregnant sheep. These studies also demonstrate the ability of $PGF_{2\alpha}$ injected extra-amniotically to diffuse widely and to initiate uterine activity.

The relative importance of the various sites of production of PGs in relation to the initiation of parturition in sheep is still unclear. The sensitivity of the myometrium to stimulation with PGs increases so markedly, following removal of the P_4 influence, that small amounts of PGs, whatever their source, may be sufficient to stimulate uterine activity. It is possible that in sheep the massive release of $PGF_{2\alpha}$ into the maternal uterine vein during the last 24 h before delivery is not essential for parturition, since most of the circulating PG is metabolized in the lungs. However, in other ruminants (e.g. goat and cows) this release of $PGF_{2\alpha}$ into the uterine vein may be of key importance since the countercurrent diffusion of $PGF_{2\alpha}$ from the utero-ovarian vein to the ovarian artery may be responsible for causing the regression of the CL of pregnancy and a decrease in maternal P_4 levels. This, in turn, would enhance the further release of PGs and render the myometrium more sensitive to the action of PGs.

9.5.6 REGULATION OF PG SYNTHESIS AND RELEASE

(a) Oestrogens

The close temporal relationship between the preparturient increases in oestrogen and $PGF_{2\alpha}$ in the utero-ovarian vein is consistent with the thesis that increasing plasma concentrations of oestrogen may stimulate the release of $PGF_{2\alpha}$ (Thorburn *et al.*, 1972a; Currie *et al.*, 1973; Challis *et al.*, 1982). Moreover, the evolution of uterine activity during the last 24 h of pregnancy (Ward, 1968; Thorburn *et al.*, 1984) coincides with the increase in maternal plasma $PGF_{2\alpha}$ (and free oestrogens), suggesting that $PGF_{2\alpha}$ may be responsible for initiating myometrial activity associated with labour. The administration of oestrogen induces premature delivery in sheep (Hindson *et al.*, 1967) and, within 24 h of treating pregnant sheep with diethylstilboestrol, concentrations of $PGF_{2\alpha}$ increase in

the maternal cotyledons and myometrium, but not in the fetal cotyledons (Liggins, 1973). These changes are associated with a marked increase in $PGF_{2\alpha}$ concentrations in the utero-ovarian vein and a 90% decrease in the threshold response to oxytocin, despite the absence of any change in peripheral or utero-ovarian P_4 concentrations.

Because oestradiol-17β was considered the primary trigger coming from the fetus to initiate $PGF_{2\alpha}$ release and parturition, oestradiol was infused into sheep during late pregnancy in amounts which produced physiological (parturient) levels in the maternal circulation and premature delivery was induced in three of four experiments (Currie *et al.*, 1973; Currie, 1974; Thorburn *et al.*, 1977a). Labour and an increased release of $PGF_{2\alpha}$ was observed in the absence of any change in circulating P_4. The concentrations of oestrogens relative to those of P_4 were considerably lower than those observed normally at parturition. Earlier in pregnancy (day 125) oestradiol (Currie *et al.*, 1973; Currie, 1974) or stilboestrol (Hindson *et al.*, 1967; Liggins *et al.*, 1973) stimulated an increase in uterine activity and $PGF_{2\alpha}$ release, but cervical relaxation and delivery did not occur.

Oestradiol given as short- or long-term (overnight) systemic infusions failed to stimulate $PGF_{2\alpha}$ release in day 15 nonpregnant ewes (Burgess *et al.*, 1990; Burgess, 1991), contrary to the results of Barcikowski *et al.* (1974), who used non-pregnant sheep bearing an autotransplanted uterus. Even doses of oestradiol 100 times greater than that used by Barcikowski *et al.* (1974) failed to induce significant increases in PGFM concentrations in the utero-ovarian vein, Burgess (1991) also found that oestradiol failed to stimulate the release of PGFM or PGE_2 from the uteri of mid-pregnant (day 60–65) and late pregnant (day 135–140) animals. These studies suggest that the action of oestradiol to stimulate PG release was blocked until day 140 of pregnancy.

It was proposed that the high P_4 concentrations throughout pregnancy may inhibit the action of oestrogen on oxytocin receptors and the PG synthetic mechanism to prevent increased PG release and that, in the days before delivery when P_4 concentrations decrease and its inhibitory effect is withdrawn, oestrogen stimulates $PGF_{2\alpha}$ release via an induction of oxytocin receptors and an increase in PG synthetic capacity (Burgess, 1991; Burgess *et al.*, 1991). The results of the early studies, in which the release of $PGF_{2\alpha}$ and uterine activity was observed, may be explained by the use of large (pharmacological) amounts of synthetic oestrogens. It is apparent from a number of studies that if sufficient oestrogen is given to increase the E/P_4 ratio to parturient levels, $PGF_{2\alpha}$ will be released irrespective of the P_4 concentrations prevailing at the time. It would seem that P_4 and oestrogen may exert opposing actions on the key enzymes in the PG synthetic pathway, i.e. PLA_2 and cyclo-oxygenase.

(b) Progesterone

In sheep, P_4 exerts a marked inhibitory influence on the myometrium and the cervix. Indeed, withdrawal of this P_4 'block' is a prerequisite for successful delivery in sheep (Thorburn, 1983; Thorburn *et al.*, 1983, 1984; Jenkin and Thorburn, 1985). The concentration of P_4 decreases 3–4 days before parturition (Nathanielsz *et al.*, 1982), before the increase in $PGF_{2\alpha}$ release (Bassett *et al.*, 1969).

The profound inhibitory action of P_4 on the ovine myometrium has been demonstrated in the non-pregnant, ovariectomized, oestrogen-primed ewe (Lye and Porter, 1978). Oxytocin and $PGF_{2\alpha}$, given in amounts sufficient to cause pronounced myometrial activity in the oestrogen-primed uterus, failed to stimulate activity in the P_4-blocked uterus, indicating that the inhibitory action of P_4 is directly on the myometrium and not simply due to the suppression of $PGF_{2\alpha}$ re-

lease. Progesterone can inhibit labour at term in sheep (Bengtsson and Schofield, 1963), although prolonged pregnancy occurred only after the administration of large amounts of P_4. The administration of P_4 in doses below 100 mg/day, sufficient to maintain physiological concentrations of P_4 (i.e. in the normal preparturient range), did not delay the initiation of parturition (Liggins *et al.*, 1973). However, high doses of P_4 (100 mg/day) were able to inhibit uterine activity and labour during fetal infusion of dexamethasone. Thorburn (1979) suggested two reasons why such large amounts of P_4 are needed to block premature or term labour in sheep. Firstly, exogenous P_4 is cleared rapidly by the placenta as a result of the increased placental blood flow in late pregnancy and the ability of the placenta to metabolize P_4. Secondly, because of the high circulating levels of oestrogen achieved at parturition due to the quantitative conversion of endogenous P_4 to oestrogen, large amounts of exogenous P_4 are needed to lower the E/P_4 ratio to preparturient levels. Subsequent studies have confirmed these ideas (Thorburn, 1983; Thorburn *et al.*, 1983; Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988). Progesterone (200 mg/day) or MPA (250 mg) significantly delayed the initiation of parturition induced by cortisol infusion. Fetal and maternal plasma PGFM levels were increased, but myometrial activity, as judged by intrauterine pressure and electromyographic recordings, did not increase (Thorburn *et al.*, 1983). These studies showed that P_4, when administered in adequate amounts, is able to inhibit uterine activity in the pregnant ewe and prevent parturition despite high levels of PGs (Thorburn *et al.*, 1983; Jenkin *et al.*, 1985a,b). These experiments also confirmed that P_4, and particularly MPA, also inhibit the release of $PGF_{2\alpha}$ from the uterus, but that supraphysiological amounts of exogenous P_4 are needed to overcome the stimulatory effects of high levels of oestrogen that are generated by the fetal cortisol induced metabolism of placental P_4 to oestrogen (Thorburn *et al.*, 1983; Jenkin *et al.*, 1985a,b; Nathanielsz *et al.*, 1988).

9.5.7 SENSITIVITY OF THE MYOMETRIUM TO $PGF_{2\alpha}$

The importance of P_4 inhibiting both myometrial activity and the stimulatory action of $PGF_{2\alpha}$ on the myometrium has been explored in the following ways. Administration of competitive inhibitors of 3β-HSD (Taylor *et al.*, 1982; Jenkin and Thorburn, 1985) causes a decrease in maternal plasma P_4 concentrations and an increase in PGFM concentrations. If maternal P_4 concentrations are decreased to sufficiently low levels, parturition occurs despite oestrogen concentrations being unchanged. However, if P_4 decreases to about 30% of the preinjection levels, despite a marked increased in PGFM concentrations in maternal plasma (up to 4 ng/ml), and a gradual increase in myometrial (EMG) activity, delivery does not occur.

The ovine myometrium when under the influence of P_4, is insensitive to PGs (Thorburn *et al.*, 1972a) and so P_4 withdrawal alone, without any associated increase in oestrogens, may therefore be enough to induce $PGF_{2\alpha}$ release and parturition. When $PGF_{2\alpha}$ was infused extra-amniotically in late pregnant ewes (132.4 ± 0.24 days' gestation) in a dose sufficient to cause $PGF_{2\alpha}$ concentrations in excess of those found in fetal and maternal plasma and in amniotic fluid at parturition (Thorburn *et al.*, 1983), there was no change in P_4 concentrations or myometrial (EMG) activity in three out of five animals and only a small increase in frequency in the other two animals. However, concomitant injection with a 3β-HSD inhibitor produced a profound decrease in maternal P_4 concentrations, a marked increase in uterine activity and parturition within 24 h.

Liggins *et al.* (1973) found that prolonged intra-aortic infusions of $PGF_{2\alpha}$ increased uterine activity and increased the sensitivity of

the myometrium to oxytocin in late pregnant ewes, but this may have been due to a longer duration and different route of administration and by the $PGF_{2\alpha}$ concentrations achieved at the myometrium. Acute administration of high doses of $PGF_{2\alpha}$ will also overcome the P_4 block and induce uterine activity in late pregnant sheep (M.D. Mitchell *et al.*, 1977b).

Local P_4 withdrawal can also be achieved by the administration of P_4 analogues (e.g. mifepristone), which can compete with endogenous P_4 for uterine binding sites but lack P_4-like activity. Administration of mifepristone to late pregnant ewes does not alter P_4 or oestrogen concentrations, and the changes in PG release and uterine sensitivity to oxytocin mimic those in preparturient animals (Burgess, 1991): basal concentrations of PGFM and PGE_2 were increased compared with values in late pregnant ewes. The ability of oxytocin to elicit uterine PGFM release was also enhanced in the treated animals (Burgess, 1991; Burgess *et al.*, 1991). These results are consistent with the proposal that P_4 withdrawal is essential in the pregnant sheep for the increased release of PGs at term.

The key role of PGs in the initiation of premature labour in sheep was clearly demonstrated by the studies of M.D. Mitchell and Flint (1978a). The administration of meclofenamate (an inhibitor of PGHS and a $PGE_{2\alpha}$ antagonist) to pregnant sheep prevented the delivery of a live fetus in response to an intrafetal dose of dexamethasone. Despite the anticipated rise in oestradiol and fall in P_4 concentrations, parturition did not occur if PG synthesis was blocked. The pretreatment contracture pattern of uterine activity persisted although the frequency of the contractures increased, possibly due to the high oestrogen levels. After cessation of meclofenamate treatment, uterine activity (labour-like contractions) commenced within 1–2 h, but $PGF_{2\alpha}$ release only occurred during second-stage labour. Presumably, only low levels of $PGF_{2\alpha}$ are needed to induce delivery in the presence of very low P_4 levels and high oestrogen concentrations.

9.5.8 OXYTOCIN

There is still no evidence that oxytocin, at least that of maternal pituitary origin, is a prerequisite for parturition in sheep. The studies of Denamur and Martinet (1961) and Bosc (1974) showed that normal parturition occurred at term after maternal hypophysectomy at days 50–134 of pregnancy, although some regrowth of neurosecretory tissue cannot be excluded in these animals (Swaab *et al.*, 1977). An oxytocin receptor antagonist which successfully blocks the action of exogenous oxytocin in pregnant sheep failed to block uterine activity at term (Jenkin *et al.*, 1990). These results suggest either that endogenous oxytocin is not responsible for causing the uterine activity associated with parturition in sheep or that, once oxytocin has exerted its action, it cannot be reversed by the administration of an antagonist. However, human fetal membranes (and/or decidua) have the potential to synthesize oxytocin (Chibbar *et al.*, 1991) and a local paracrine (and/or autocrine) action by oxytocin in the membranes, endometrium and decidua of the sheep could allow for the local generation of PGs and a direct action of oxytocin on the myometrium, the release of oxytocin being mediated by local changes in the E/P_4 ratio.

Glatz *et al.* (1981) concluded that oxytocin did not stimulate the onset of labour in sheep, but did facilitate delivery of the fetus and placenta by inducing forceful uterine contractions, confirming suggestions that oxytocin plays an important role during second- and third-stage labour in the sheep (Currie *et al.* 1973). Vaginal distension provokes the reflex release of oxytocin (Ferguson, 1941; Debackere *et al.*, 1961; Roberts and Share, 1968) and $PGF_{2\alpha}$ (Flint *et al.*, 1974b) and P_4 can inhibit this response in nonpregnant ewes (Roberts and Share, 1968).

When the vagina is distended in late pregnant ewes, an increase in plasma oxytocin concentrations is followed 1 min later by an increase in PGF concentrations (Flint *et al.*, 1975); the response increases as delivery approaches and is most pronounced in the immediate post-parturient animal, indicating that the sensitivity of the posterior pituitary to vaginal distension in the periparturient ewe is influenced by the prevailing P_4 concentration (Flint *et al.*, 1975; M.D. Mitchell *et al.*, 1977b). The infusion of oxytocin into late pregnant ewes also causes the release of $PGF_{2\alpha}$ into the utero-ovarian vein, and this response increases towards term (M.D. Mitchell *et al.*, 1975; Burgess, 1991), paralleling the increasing ability of oxytocin to cause uterine contractions nearer delivery (Hindson *et al.*, 1969). The recent studies of Burgess (1991) have confirmed and extended the earlier observations of Mitchell *et al* (1975). She found that oxytocin stimulated the uterine release of PGFM and that the response was greater in preparturient (day 140 term) than in late pregnant (130–135 days) ewes and concluded that the capacity of the PG secretory system to respond to oxytocin increased markedly near term. The increased response to oxytocin is dependent not only on the plasma concentration of oxytocin but also on the affinity and concentration of its receptors in the target tissue (Alexandrova and Soloff, 1980a; Fuchs *et al.*, 1983b). In endometrial explants from non-pregnant ewes, the ability of oxytocin to stimulate $PGF_{2\alpha}$ release was correlated with the oxytocin receptor concentration, and the oxytocin receptor concentration varied directly with the E:P_4 ratio, high levels being found at oestrus (Roberts *et al.*, 1976). It seemed likely that the increased $PGF_{2\alpha}$ secretory response to oxytocin preceding delivery was due to an increased concentration of oxytocin receptors in the endometrium and maternal placenta resulting from the progressive increase in the E/P_4 ratio (Burgess, 1991). Intra-arterial oxytocin injection significantly increases the release of PGFM into the utero-ovarian vein during mid- and late pregnancy in the sheep, but oxytocin fails to stimulate the release of PGE_2 (Burgess *et al.*, 1989; Burgess, 1991), indicating that an endogenous inhibitor of PGE_2 release may be present in the ovine endometrium during late pregnancy. Fetal injection of oxytocin during late pregnancy fails to stimulate PGE_2 release (Jenkin *et al.*, 1989), thus supporting the contention that the PGE_2-secreting cells do not possess oxytocin receptors. These results provide further evidence that PGE_2 and $PGF_{2\alpha}$ are synthesized in different sites and suggest that the massive release of $PGF_{2\alpha}$ during labour is mainly derived from the endometrium, which is highly responsive at that time to oxytocin.

9.5.9 SUMMARY

This section should be read in conjunction with Chapter 10, this volume, on the development of the fetal hypothalamo-pituitary–adrenal axis. The studies described emphasize the importance of P_4 in the maintenance of pregnancy in the sheep. P_4 is essential for maintaining myometrial quiescence during gestation and a decrease in maternal P_4 concentrations (i.e. the removal of the so-called P_4 block) is a prerequisite for dilation of the cervix and the explosive myometrial activity associated with labour. The decrease in maternal P_4 concentrations is caused by increasing concentrations of cortisol in the fetal circulation acting on the placenta to induce the enzyme 17α-hydroxylase. In the late pregnant sheep, the placenta is the major site of P_4 production and the increased activity of the 17α-hydroxylase increases the metabolism of P_4 to oestrogens, and thus decreases the plasma levels of P_4, while increasing those of oestrogens, resulting in a marked increase in the E/P ratio. This profound change in the hormonal environment of the myometrium enhances its sensitivity to agonists such as $PGF_{2\alpha}$, PGE_2 and oxytocin.

The decrease in P_4 concentration (and the increase in oestrogen levels) not only increases the sensitivity of the myometrium to $PGF_{2\alpha}$ and oxytocin, but also favours their release. As P_4 concentrations fall, $PGF_{2\alpha}$ release from the maternal placenta, endometrium and fetal membranes increases. Some $PGF_{2\alpha}$ diffuses directly from the endometrium and fetal chorioallantois to the myometrium, whereas some is transported via the systemic circulation to the myometrium, the relative importance of the two pathways has yet to be determined.

Oxytocin is released from the fetal posterior pituitary in second-stage labour by the stretching of the cervix by the fetal head (the so-called Ferguson reflex) and by the high concentration of $PGF_{2\alpha}$ released from the uterus at this time. Oxytocin, apart from stimulating uterine activity directly, causes the further release of $PGF_{2\alpha}$. The increase in the E/P ratio favours the release of oxytocin. Recent studies suggest that oxytocin may be synthesized and released locally from the membranes and myometrium, and can therefore act directly on the myometrium. These findings, if confirmed, would explain how oxytocin may play a role in the initiation of parturition without being detected in the maternal circulation.

The recent findings of increasing production of PGE_2 during the last third of gestation, adds a new dimension to the discussion of the initiation of parturition. The increase in placental PGE_2 production appears to be under fetal pituitary control. It has been suggested that the increasing concentration of PGE_2 in the fetal circulation may play an important role in maintaining high fetal concentration of cortisol, and therefore in initiating the decrease in maternal P_4 concentrations. Large amounts of PGE_2 are secreted into the maternal circulation, and since it is not completely cleared by the lungs, increasing concentrations of PGE_2 are found in the maternal arterial circulation during the last few weeks of gestation. This arterial PGE_2 may act on the cervix to initiate cervical ripening and on the mammary gland to initiate lactogenesis; in both cases P_4 withdrawal may be an important element. The PGE_2 in the arterial circulation also provides a continuous stimulus to the myometrium and may be responsible for the appearance of the uterine 'contractures' observed during the latter part of pregnancy in the ewe. These 'contractures' may represent the effect of a P_4 -blocked myometrium responding to a potent challenge from PGE_2 in the arterial circulation. When P_4 concentrations decrease in the days preceding parturition, the PGE_2 in the arterial circulation may become a more potent stimulus to the myometrium, causing a more profound increase in myometrial activity and the initiation of labour. PGE_2 may also provide a stimulus for the release of oxytocin from the posterior pituitary and, possibly, the membranes and myometrium.

Thus, the initiation of parturition in sheep is the successful accomplishment of a continuous dialogue between the fetus and its placenta so that the fetus is delivered at an appropriate time, and an appropriate size, with its key organ systems mature and fully prepared for post-natal life.

9.6 GOAT

9.6.1 INTRODUCTION

Compared with the sheep and pig, there is a relative paucity of information on the initiation of parturition in goats. This is due to both a lack of industry funds and importance of the goat industry in most countries.

From a scientific point of view, the goat is an excellent experimental animal and is representative of animals which are dependent on the corpus luteum of pregnancy as a source of progesterone. In this species, the continued function of the corpus luteum is essential for the maintenance of pregnancy and the factors (luteotrophins) regulating its function are clearly of great importance. Early

in pregnancy the maternal pituitary hormones, prolactin and luteinizing hormone (LH) play this role, whereas in the latter part of pregnancy the nature of the luteotrophins is still a matter requiring further study, although several placental factors have been proposed including caprine placental lactogen (cPL), prostaglandin E_2 (PGE_2), a putative caprine chorionic gonadotrophin (cCG) and oestrogens. The goat is also a classical example of a species in which P_4 inhibits myometrial activity throughout pregnancy and its withdrawal (i.e. a decrease in maternal plasma P_4 concentrations) is an essential prerequisite for the onset of labour.

In both the goat and sheep, a major decrease in maternal plasma P_4 concentrations is needed to remove the inhibitory effects of P_4 on the myometrium and to allow increased myometrial activity and delivery of the fetus. In the sheep, the placenta is the major site of progesterone production during late pregnancy, whereas in the goat the corpus luteum serves this function.

As in sheep, activation of the fetal pituitary–adrenal axis appears to play a primary role in the initiation of parturition in the goat but, unlike sheep, in which fetal plasma cortisol acts directly on the placenta to decrease placental P_4 to the low levels necessary for parturition (at 150 days of gestation), fetal cortisol acts indirectly to cause regression of the maternal CL of pregnancy and thereby reduces circulating P_4 concentrations (by increaseing the level of 17α-hydroxylase). In the goat, fetal cortisol must act indirectly to cause regression of the CL of pregnancy, therby reducing circulating P_4 concentrations. Oestrogen at low levels may exert a direct trophic action on the CL, whereas at high concentrations, and particularly when P_4 concentrations start to fall, oestrogen exerts a luteolytic action by stimulating the release of $PGF_{2\alpha}$ from the endometrium and maternal placenta. Thus, the fetus, by means of its placenta and the secretion of oestrogen, initially maintains the CL, P_4 secretion and the pregnancy and then, by increasing oestrogen secretion, contributes to the regression of the CL and the termination of the pregnancy.

It has also been proposed that the initial decrease in P_4 concentrations may result from withdrawal of luteotrophic support of the corpus luteum due to a cortisol-induced decrease in cPL production by the placenta. The initial decrease in progesterone concentrations may then precipitate the release of luteolytic amounts of $PGF_{2\alpha}$. Recently, it has been shown, both in sheep and goats, that the placenta secretes increasing amounts of PGE_2 into the fetal and maternal circulations during the last third of gestation. In the goat, PGE_2 may play an important role as a luteotrophin during the last third of gestation until the demise of the corpus luteum is brought about by the release of large amounts of $PGF_{2\alpha}$ from the 'maternal' placenta during the one to two days before delivery.

9.6.2 THE FETAL PITUITARY–ADRENAL AXIS

The infusion of synthetic ACTH into fetal goats induces premature parturition (Thorburn *et al.*, 1972a), the maternal endocrine changes during which are similar to those before parturition at term (Currie *et al.*, 1973; Currie and Thorburn, 1977a,b). The concentration of corticosteroids in fetal plasma increases gradually between 4 and 11 days before term and then more rapidly during the last 4 days of pregnancy (Currie *et al.*, 1973; Currie and Thorburn, 1977a,b). Pregnancy was prolonged beyond term after ablation of the fetal pituitary by electrocoagulation (Currie and Thorburn, 1977a).

These studies led to the suggestion that a raised cortisol concentration in the fetus caused the onset of labour through effects on maternal steroid concentrations (Currie and Thorburn, 1977a). Infusion of oestradiol-17β into pregnant goats caused luteal regression (Currie and Thorburn, 1976) and, because

the concentration of oestrogen in maternal plasma rises before P_4 falls, it seemed likely that the primary effect of increased fetal adrenal activity in the goat was to induce increased oestrogen synthesis (Currie and Thorburn, 1977a).

Flint *et al.* (1978) proposed two mechanisms by which increased fetal adrenal activity might increase placental oestrogen biosynthesis: (1) that the fetal adrenal not only secretes cortisol but also androgens, which are aromatized to oestrogens by the placenta and (2) that fetal cortisol induces in the placenta the enzymatic capacity to synthesize oestrogens *de novo*. Intrafetal infusion of dexamethasone induces premature parturition in the goat (Flint *et al.*, 1978), indicating that a glucocorticoid is the only product of the adrenal essential for this process. The infusion of dexamethasone suppresses the fetal pituitary–adrenal axis as judged by the low fetal cortisol concentrations. The fetal glucocorticoids induce 17α-hydroxylase activity in the goat placenta and thus play an important role in initiating parturition in this species.

It has been proposed that a luteotrophic complex may maintain the CL of pregnancy in the goat (Thorburn, 1979) and that withdrawal of this trophic support may lead to a fall in maternal P_4 levels which, in turn, may enhance the release of $PGF_{2\alpha}$ from the maternal placenta (Thorburn, 1979; Thorburn and Challis, 1979). The placental secretion of placental lactogen (cPL), which forms part of this putative luteotrophic complex, would appear to be regulated by the levels of fetal cortisol (Currie *et al.*, 1977). During the last 15 days before delivery when fetal cortisol concentrations were rising, cPL values progressively decreased when synthetic ACTH was infused into the fetus. Maternal cPL levels decreased more abruptly. Thus, increased activity of the fetal pituitary–adrenal axis may switch off the production of cPL and decrease the trophic support of the CL. The consequent decrease in P_4 concentrations, together with the increased oestrogen synthesis resulting from activation of the fetal pituitary–adrenal axis, may produce a sufficient change in the E/P_4 ratio in the maternal placenta to cause the release of small amounts of $PGF_{2\alpha}$ and to initiate luteal regression. As P_4 concentrations decrease further, $PGF_{2\alpha}$ release is enhanced causing the further demise of the CL. This second mechanism may prove to be most important because the preparturient increase in oestrogen biosynthesis, as judged by plasma oestradiol-17β concentrations, is not marked (Flint *et al.*, 1978).

9.6.3 MAINTENANCE OF THE CORPUS LUTEUM OF PREGNANCY

The goat is representative of many species in which the CL is the major site of P_4 production throughout pregnancy. Parturition is preceded by regression of the CL and a decrease of the concentration of P_4 in the maternal plasma (Meites *et al.*, 1951; Linzell and Heap, 1968; Irving *et al.*, 1972a; Thorburn and Schneider, 1972). Removal of the CL at any time during pregnancy results in abortion, but the pregnancy can be maintained by replacement therapy with P_4 (Meites *et al.*, 1951; Irving *et al.*, 1972b) or MPA (Sheldrick *et al.*, 1980, 1981). Luteal regression and premature parturition can be induced by administration of $PGF_{2\alpha}$ to the mother (Currie and Thorburn, 1973). Premature parturition can also be induced by maternal administration of oestradiol-17β, which probably acts by stimulating uterine production of PGs (Currie and Thorburn, 1976). An understanding of the initiation of parturition in the goat therefore requires a knowledge of factors maintaining the CL during pregnancy and those causing its regression at term.

Goats, hypophysectomized between 38 and 120 days of pregnancy or pituitary stalk sectioned between 44 and 120 days, aborted 3–9 days later, indicating that the maternal pituitary gland is essential for the maintenance of the CL (Cowie *et al.*, 1963). Pregnancy

was maintained in goats hypophysectomized after day 90, by the administration of P_4 (20 mg/day) or LH (1 mg/day) but not prolactin (1–5 mg/day). When LH treatment was stopped, P_4 concentrations fell and the animals aborted. Because LH treatment was able to maintain P_4 secretion and pregnancy in two out of four goats hypophysectomized at day 60, Buttle (1978) concluded that, after day 90 of gestation, LH but not prolactin was an essential part of the luteotrophic complex. Buttle (1978, 1983) proposed that cPL could replace prolactin for CL maintenance in late pregnancy.

Malecki (1989) suggested that surgical stress, trauma and preoperation fasting, rather than removal of the pituitary hormones, may have resulted in abortion. Other workers have reported a tendency for goats to abort when subjected to surgical (Flint *et al.*, 1978) and environmental stress (van Heerden, 1961; van Rensburg, 1971). In an attempt to protect the pregnant goat against surgical stress, and assuming that luteolysis occurred in association with surgery, P_4 was administered for the first 9 days after surgery. Despite the P_4 treatment, abortions still occurred at 13.5–17.5 days after surgery, suggesting that regression of the CL had occurred around the time of surgery. The ability of administered LH to prevent abortion in hypophysectomized goats in the studies of Buttle (1978, 1983) was presumably due to the maintenance of P_4 concentrations by the exogenous LH. While this study demonstrated that LH was luteotrophic it did not conclusively demonstrate that LH was essential for pregnancy maintenance.

Although the exact mechanisms of stress-induced abortions are not known in the goat, some studies provide a clue to possible mechanisms involved. The infusion of synthetic ACTH into a pregnant goat not only produced a marked increase in maternal cortisol concentrations but also a significant increase in fetal cortisol values (Thorburn *et al.*, 1972a). If maintained for some days, high fetal cortisol concentrations are likely to induce premature parturition in the goat. While investigating post-surgical abortion in mares, Silver *et al.* (1979b) noted that presurgical food restriction caused an elevation in maternal $PGF_{2\alpha}$ concentrations and that this in turn led to luteal regression and premature labour. In other species, LH has been shown to antagonize the luteolytic action of $PGF_{2\alpha}$, and this may explain the protective action of the LH treatment in the studies of Buttle (1978, 1983).

In the studies of Malecki (1989), one goat hypophysectomized at day 84 of pregnancy was able to maintain the pregnancy and P_4 production within the range expected for unoperated goats until near term. Although hypophysectomy in this goat was not anatomically complete and prolactin was present in the peripheral circulation, LH could not be detected in any blood sample after hypophysectomy or after infusion of 200 mg LHRH on two separate occasions. It would appear that in this animal hypophysectomy was functionally complete with respect to LH, and Malecki (1989) concluded that LH may not be essential for the maintenance of pregnancy after day 84 of gestation in the goat.

To avoid the side-effects of surgical hypophysectomy, Malecki *et al.* (1987) used passive immunization against LH to study the effect of LH deprivation during pregnancy: infusion of antiserum against oLH in goats between 13 and 120 days of gestation resulted in abortions in five out of five goats immunized up to day 35 and in 24% of goats between days 42 and 120 of gestation. All goats immunized after day 35 of gestation displayed a significant drop in peripheral plasma P_4 levels regardless of whether abortion occurred. This indicates that maternal pituitary LH is essential for pregnancy maintenance up to at least day 35 of pregnancy, although LH still has a substantial luteotrophic role between days 42 and 120, as suggested by Currie and Thorburn (1974), who studied the effect on CL function of hysterec-

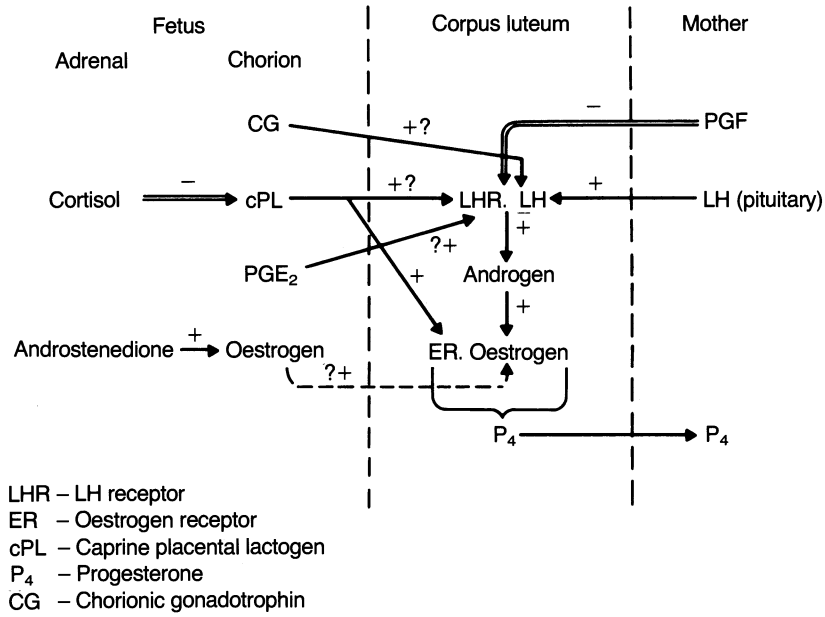

Figure 9.11 The putative mechanisms involved in trophic regulation of the CL of pregnancy in the goat. (Modified from Thorburn, 1978.)

tomy (between days 30 and 50) in pregnant goats.

The evidence in favour of the luteotrophic action of cPL has been summarized by Malecki (1989) as follows:

1. cPL becomes detectable by radioreceptor assays in the peripheral plasma at about day 50 of gestation and reaches peak levels at days 110–130 (Currie *et al.*, 1977).
2. An increase in peripheral P_4 concentrations occurs between days 30 and 50 of pregnancy (Thorburn and Schneider, 1972) which corresponds to the appearance of cPL in the maternal circulation.
3. Hysterectomy of goats between 30 and 50 days of pregnancy results in a decline in peripheral P_4 to less than half presurgery levels, suggesting that a luteotrophin originating from the conceptus has been removed (Currie and Thorburn, 1974).
4. Two out of four goats hypophysectomized at day 60 of gestation aborted despite replacement therapy with 1 mg of LH per day, suggesting that cPL production at this stage of pregnancy may be too low to maintain CL function.

Based on the observations of Currie and Thorburn (1974) and Buttle (1978, 1983) it was suggested that cPL and LH may form a luteotrophic complex around day 50 of pregnancy in the goat (Figure 9.11).

A more detailed description of a possible caprine luteotrophic complex is given by Thorburn (1979), but there is still no direct evidence that cPL plays a luteotrophic role in the pregnant goat and cPL has not yet been isolated and purified. It would be of interest to repeat the passive immunization experiments of Malecki *et al.* (1987) using antiserum to cPL as well as oLH.

(a) Chorionic gonadotrophin

Material showing activity in an hCG radioimmunoassay and bovine granulosa cell radioreceptor assay has been detected (prob-

ably a large molecular weight glycoprotein) in placentas from goats at 61, 69, 74 and 143 days of pregnancy (Malecki, 1989). This material did not cross-react in an LH radioimmunoassay, suggesting that it was not LH of pituitary origin bound to cotyledonary tissue.

An hCG-like material was also tentatively identified in the urine of pregnant (60–140 days' gestation) but not non-pregnant goats. The urinary CG-like material could be the same as the high molecular weight form identified in the placenta because the glycosylated side chains could be removed to permit filtration by the kidney. It was difficult to estimate accurately the level of CG-like material in goat placental tissue as the degree of cross-reaction of the hCG RIA in the radioreceptor assay was unknown, but the quantity appeared to be very low compared with that found in the placentas of other species (Malecki, 1989). However, Malecki (1989) suggests that cPL together with CG (i.e. a placental LH-like substance) may form a luteotrophic complex, together with maternal pituitary LH and prolactin, during the second half of pregnancy in the goat.

(b) Oestrogens

There is strong evidence that oestrogens are luteotrophic in some species, e.g. rabbit (Bill and Keyes, 1983; Gadsby *et al.*, 1983) and rat (Bugdanove, 1966). There appears to be no direct evidence of a luteotrophic action of oestrogens in the pregnant goat. In fact, the administration of oestradiol-17β to pregnant goats provoked $PGF_{2\alpha}$ release from the uterus and caused luteolysis with subsequent abortion (Currie and Thorburn, 1976), although oestrogens of fetoplacental origin may play a luteotrophic role during pregnancy. Ovariectomy during pregnancy maintained by a synthetic progestagen did not alter oestradiol-17β concentrations, indicating that the conceptus and not the ovary is the major source of oestrogen (Currie and Thorburn, 1977a,b; Sheldrick *et al.*, 1980). It has not yet been demonstrated whether oestrogen is part of the luteotrophic signal from the conceptus.

(c) Prostaglandin E_2

PGE_2 is the major PG produced by the goat placenta and membranes (M.D. Mitchell *et al.*, 1978f). Like the sheep (Fowden *et al.*, 1987) the goat placenta produces increasing amounts of PGE_2 (G.D. Thorburn, unpublished) and it is possible that placental PGE_2 may provide a luteotrophic stimulus for the CL of pregnancy during the last third of gestation. (For discussion on the potential luteotrophic action of PGE_2 in the pig see section 9.4.3.)

9.6.4 OESTROGEN AND THE FETAL PITUITARY-ADRENAL AXIS

In the goat, unconjugated oestrogens increase steadily in the maternal plasma throughout pregnancy before undergoing a more rapid increase over the last 4–5 days before delivery (Challis and Linzell, 1971; Thorburn *et al.*, 1972a; Umo *et al.*, 1976; Currie and Thorburn, 1977a; Flint *et al.*, 1978). The major oestrogens in maternal plasma are oestrone and oestradiol-17α, both considered relatively weak biologically. However, $E_{2\beta}$ can be formed from oestrone in maternal tissues (Challis and Linzell, 1973) and is probably the active oestrogen, plasma concentrations following a similar time course throughout pregnancy (Challis and Linzell, 1971; Flint *et al.*, 1978; Meinecke-Tillmann *et al.*, 1986). The concentrations in blood of unconjugated oestrone and oestradiol-17α are approximately one-third those of the corresponding sulphoconjugates (Thorburn *et al.*, 1972a; Currie *et al.*, 1973). Oestradiol-17α does not induce labour (Currie and Thorburn, 1976).

Active aromatase activity is present in the

goat placenta (Ainsworth and Ryan, 1970) and the progressive increase in maternal oestrogen concentrations could result from placental aromatization of C_{19} precursors derived from the fetal adrenal gland (Thorburn and Challis, 1979) because infusion of synthetic ACTH into fetal goats causes an increase in fetal and maternal oestrogen levels and premature parturition (Thorburn *et al.*, 1972a). These results could be explained in at least two ways: (1) that the fetal adrenal secretes not only corticosteroids, but also androgens, which are aromatized to oestrogen by the placenta; and/or (2) that the fetal corticosteroids induce in the placenta the enzymes needed to synthesize oestrogens *de novo*. The studies of Flint *et al.* (1978) suggested that increased production of fetal adrenal androgens is not essential for the increased oestrogen production before delivery and that, as in the sheep, high fetal levels of cortisol induce or activate placental steroid 17α-hydroxylase which increases the endogenous production of C_{19} precursors in the placenta for oestrogen biosynthesis.

In late pregnancy the goat placenta may produce small amounts of P_4 (Sheldrick *et al.*, 1980), but substantial amounts of oestrogens are secreted and plasma levels concentrations increased progressively from day 40 of pregnancy onwards; these levels are considerably higher than those found in sheep. The goat placenta also produces substantial amounts of 5β-pregnane-3α,20α-diol (Sheldrick *et al.*, 1981), which increases in the peripheral circulation between days 40 and 140 of gestation, reaching levels of 80 to 100 ng/l before decreasing rapidly before term. The main circulating products of placental steroid synthesis are therefore 5β-pregnane-3α,20α-diol, oestrone, oestradiol-17α and their sulphoconjugates. Considering the dramatic decrease in the plasma concentrations of 5β-pregnane-3α,20α-diol before parturition it is surprising that there is not a more dramatic increase in oestrogen values at this time. The possibility must exist that some other product which is synthesized in considerable amounts has been missed.

In the studies of Sheldrick *et al.* (1981), it had been assumed that the precursor of 5β-pregnane-3α,20α-diol synthesis was P_4 and that P_5 was converted by 3β-HSD to P_4, which was then reduced by 5β-reductase to 5β-reduced derivatives. However, the goat may lack a placental 3β-HSD and possess a 5β-reductase instead, leading to direct reduction of P_5 by 5β-reductase before reduction by 20α-hydroxylase through to the 20α-ol derivative. However, the major metabolite found in the circulation of the pregnant goat was the 3α-ol derivative (Sheldrick *et al.*, 1981) suggesting that it may be derived from P_4 (perhaps produced in the CL and metabolized in the placenta) or that P_5 is converted to the 3α derivative before 5β reduction.

In vitro studies (Wango *et al.*, 1988) showed that the goat does not synthesize P_4 but converts labelled P_5 to 5β-pregnanediol, which was the major metabolite. This direct route of metabolism is consistent with the hypothesis that a major difference between the sheep and the goat is that the sheep placenta contains 3β-HSD, which allows the conversion of P_5 through to P_4 (section 9.5.2), whereas in the goat there is a relative deficiency of this enzyme and the goat placenta contains a 5β-reductase which converts P_5 through to the inactive 5β-reduced derivatives. There is no information on whether the goat placenta secretes P_5, and the circulating concentrations of P_5 and P_5 sulphate in the goat are not known, but they are likely to be high.

Sheep and goats are sufficiently close phylogenetically to allow interspecies crosses to survive until approximately day 40 of pregnancy (Hancock *et al.*, 1968). This may be because the goat placenta is unable to synthesize C_{21} steroids, although this appears unlikely in view of the low uterine production of P_4 in ovariectomized or luteectomized goats in which pregnancy is maintained with MPA (Sheldrick *et al.*, 1980). However, the main difference between the

two species may relate to the presence (or not) of placental 5β-reductase or the relative activities of 5β-reductase to 3β-HSD. In species which contain 5β-reductase and metabolize P_5 to inactive metabolites, it would seem essential for the CL to remain functional and secrete significant amounts of P_4. There is an added need for the placenta to secrete luteotrophic substances to maintain the CL functional for the rest of pregnancy.

Although glucocorticoids may increase 3β-HSD activity and *de novo* placental P_4 biosynthesis from P_5, Flint *et al.* (1978) failed to find any conversion of androstenedione to oestradiol-17β by placental explants of the goat and suggested that the raised levels of oestradiol-17β in the maternal circulation at term may not be placental in origin. The mammary gland of the goat produces oestradiol-17β in late pregnancy (Maule Walker and Peeker, 1978), accounting for over 90% of the increase in peripheral plasma concentrations of oestradiol-17β near term. It is possible that oestrone or oestradiol-17α produced by the uterus may be converted into oestradiol-17β by mammary 17-dehydrogenase.

9.6.5 LUTEOLYSIS

Oestrogens are luteolytic in pregnant goats. Infusion of oestradiol-17β, but not oestradiol-17α, into late pregnant goats resulted in PGF release into the uterine vein, leading to regression of the CL (Currie and Thorburn, 1976). It was therefore proposed that increased oestrogen biosynthesis by the fetal placenta was responsible for the initiation of parturition of the goat (Currie and Thorburn, 1977a; Thorburn, 1979; Thorburn and Challis, 1979): oestradiol-17β produced by the fetal placenta would traverse the placental barrier and act on the oestrogen receptors in the maternal placenta which respond by increased production of $PGF_{2\alpha}$. Currie and Thorburn (1976, 1977a) proposed that oestrogen increased the number of oxytocin receptors, whereas P_4 acted at the nuclear locus to repress synthesis of oxytocin receptors, as occurs in sheep (Roberts *et al.*, 1976).

When premature parturition was induced with oestradiol-17β, the placenta and membranes were delivered within 12 h of the fetus, perhaps because of the oestrogen causing breakdown of the lysosomes and the release of hydrolase enzymes, causing dissolution of the maternal placenta and placental separation (Currie and Thorburn, 1977a).

Activation of fetal pituitary–adrenal function may provide a stimulus to the final increase in oestrogen concentrations before parturition (Currie *et al.*, 1973; Currie and Thorburn, 1977a; Thorburn *et al.*, 1977a,b; Thorburn, 1979). Premature parturition resulting from ACTH infusion to the fetus is associated with elevated oestrogen concentrations in fetal and maternal plasma and increased release of $PGF_{2\alpha}$ into the uterine vein (Thorburn *et al.*, 1972a; Currie and Thorburn, 1977a), suggesting that oestrogens synthesized in the fetal cotyledons crossed the placental barrier to stimulate $PGF_{2\alpha}$ production by the maternal cotyledons.

When CL regression is induced by the infusion of $PGF_{2\alpha}$ into the pregnant goat during late pregnancy, plasma P_4 concentrations decline, and 30–40 h later there is a major release of endogenous $PGF_{2\alpha}$ into the uteroovarian vein. The concentration of $PGF_{2\alpha}$ is maximal at the time of delivery of the kid (Currie and Thorburn, 1973). Similarly, endogenous $PGF_{2\alpha}$ is released 24–36 h after CL regression induced during the mid-luteal phase of the sheep oestrous cycle by $PGF_{2\alpha}$ analogues (Challis *et al.*, 1976b). In both instances P_4 withdrawal has been proposed as the stimulus for the increase in $PGF_{2\alpha}$, which is consistent with the major action of P_4 in suppressing PG release. Abortion in goats after ovariectomy or luteectomy can be prevented by exogenous P_4 (Meites *et al.*, 1951; Irving *et al.*, 1972b). When P_4 was administered to ovariectomized pregnant goats in a manner that caused large fluctuations in the

plasma P_4 concentrations, there was a significant increase in $PGF_{2\alpha}$ concentrations in the uterine vein with each fall in P_4 (Thorburn *et al.*, 1972a). After cessation of treatment, the P_4 concentrations fell and $PGF_{2\alpha}$ values rose to a plateau of about 7 ng/ml that was maintained for about 10 h before a further large increase occurred with labour itself. These experiments clearly indicate that P_4 suppresses the release of $PGF_{2\alpha}$ and suggested that the release mechanism is labile and quickly responds to variations in the plasma P_4 concentration (Thorburn *et al.*, 1972a; Currie and Thorburn, 1977b).

In contrast, when premature parturition was induced with oestradiol-17β the goats displayed striking mammary engorgement which was evident within 24 h of the start of treatment. The lactogenic response seen in these animals is in contrast to the agalactia observed in premature parturition induced with luteolytic doses of $PGF_{2\alpha}$ but is similar to that observed with fetal ACTH infusion (Thorburn *et al.*, 1972a; Currie and Thorburn, 1976). These doses of oestradiol-17β presumably stimulated the release of prolactin from the maternal pituitary which acted with the elevated oestradiol and low P_4 concentrations to give the observed lactogenic response.

9.6.6 SUMMARY

It seems likely that the following sequence of events is involved in the initiation of parturition in goats. Activation of the fetal hypothalamo-pituitary–adrenal axis causes a progressive increase in fetal cortisol concentrations during the last 10 days before delivery. Activation of the fetal adrenal may also increase the plasma concentrations of androstenedione (and other substrates) for aromatization to oestrogens by the placenta. Fetal cortisol induces 17α-hydroxylase activity in the placenta, facilitating the conversion of endogenous P_5 to oestrogens within the placenta (and chorion?). Oestrogens, in turn, act locally in the placenta (and maternal endometrium?) to stimulate increased production of $PGF_{2\alpha}$, which is released into the uterine vein. The countercurrent mechanism facilitates transfer of $PGF_{2\alpha}$ from the utero-ovarian vein to the ovarian artery and the $PGF_{2\alpha}$ initiates regression of the CL. The initial decrease in P_4 enhances the release of further $PGF_{2\alpha}$, ensuring rapid and complete regression of the CL (Figure 9.12).

High fetal cortisol concentrations also decrease maternal PL values and may thus reduce trophic support for the CL and lower maternal P_4 concentration. The decrease in P_4 levels may enhance the release of $PGF_{2\alpha}$ from the maternal placenta, and might be expected to work in concert with the high oestrogen values. The increase in the E/P_4 ratio in the placenta may increase the number of oxytocin receptors. The change in the E/P_4 ratio in maternal plasma, together with hypothalamic stimulation via the Ferguson reflex, would ensure release of oxytocin from the maternal posterior pituitary, the oxytocin causing a massive release of $PGF_{2\alpha}$ during labour.

The mammary gland also produces oestradiol-17β in late pregnancy and may contribute to the increase in the E/P_4 ratio and provide a further stimulus for $PGF_{2\alpha}$ release. The myometrium is exposed to $PGF_{2\alpha}$ indirectly from the maternal placenta and directly by diffusion from the endometrium. The relative importance of these two routes has not been established. The sensitivity of the myometrium to $PGF_{2\alpha}$ increases with the fall in P_4 concentrations. It is not yet known whether PGE_2 exerts a stimulatory action on the caprine myometrium or the exact site of its synthesis. However, using the sheep analogy, PGE_2 is probably synthesized in the chorion and fetal placenta and may reach the myometrium by the same route as $PGF_{2\alpha}$.

9.7 CATTLE

The major endocrine changes associated with parturition (days 277–290) in cattle are mostly

clearly defined. Some features are similar to those of other ruminants such as the sheep and goat, but differences are apparent particularly in the source of P_4.

9.7.1 ROLE OF THE FETUS

Studies of prolonged pregnancy in cattle yielded the first clear evidence of an essential role for the fetus in the initiation of parturition in any species. Genetic analysis of inbred stock (Jasper, 1950; Kennedy *et al.*, 1957) showed that, although the patterns of inheritance are similar, there are at least two distinct genetically controlled syndromes (Kennedy *et al.*, 1957, 1967). Since a cow that is heterozygous for the mutant gene may have normal or abnormal pregnancies in any sequence when bred to a heterozygous bull, Holm (1967) postulated that the bovine fetus

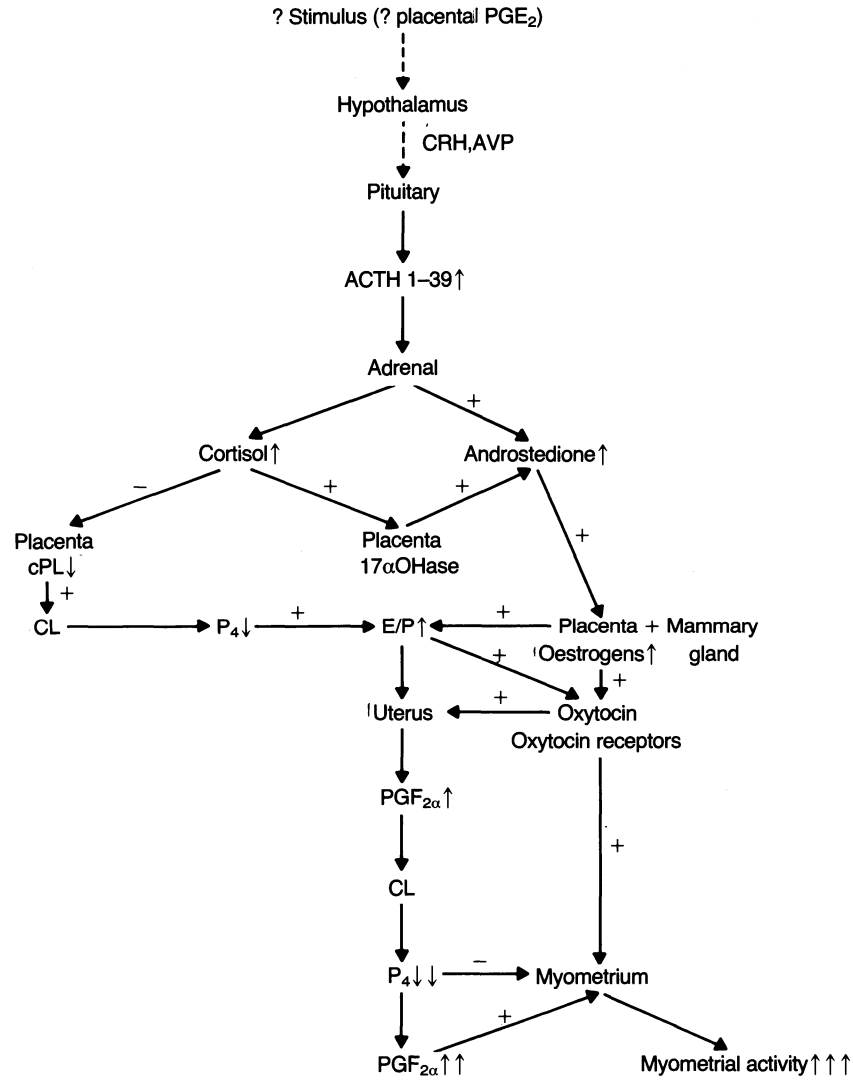

Figure 9.12 The pathways involved in the initiation of parturition in the goat.

interacting with the maternal organism controls the length of its intrauterine life. The circumstantial evidence derived from study of the malformed calves points strongly to the anterior pituitary as the primary defect causing prolonged pregnancy, with adrenocortical insufficiency as the next step in the chain. Although the roles of the fetal pituitary and adrenal have not been investigated directly by experimental ablation in cattle, much evidence is available to support the hypothesis that parturition in cattle is initiated, as it is in sheep, by activation of the fetal pituitary–adrenal system. The concentration of cortisol in fetal plasma rises prepartum, but there is some disagreement as to the magnitude of the increase. Comline *et al.* (1974), who studied 10 chronically cannulated fetuses, reported that the concentration of cortisol was usually <5 ng/ml before 250 days of gestation, rising to about 10 ng/ml in fetuses 7–10 days before parturition and to a mean value of 61 ng/ml within 1 h of birth, which was premature at a mean of 264 days. In the series of five animals described by Hunter *et al.* (1977), values up to 7 days before parturition were similar to those reported by Comline *et al.* (1974), but the higher mean prepartum value (74 ng/ml) was attributed to delivery at term. Takeishi *et al.* (1989) reported similar findings in late pregnancy but did not sample close to term. However, the concentration of 88 ng/ml found in neonates immediately after delivery agrees with that of 94 ng/ml found by Comline *et al.* (1974). ACTH concentrations rise from 60–70 pg/ml at 210 days to 240 pg/ml at 240 days and to 400 pg/ml at 270 days (Takeishi *et al.*, 1989), but values in the neonates fell to 180 pg/ml, a finding interpreted as evidence that the prepartum increase in cortisol concentrations may be due to an increase in the sensitivity of the adrenal to ACTH.

Intramuscular or intravenous injections of ACTH into chronically cannulated fetuses but not the mother cause premature parturition (Welch *et al.*, 1973) after a delay of about 6 days. The concentration of cortisol increases during treatment in a pattern similar to that at normal term and is associated with changes in maternal plasma oestrogen and P_4 concentrations that mimic the normal pattern. Fetal treatment with cortisol has been investigated in only one animal (Comline *et al.*, 1974): 100 mg of cortisol were injected four times daily intravenously and premature parturition at 257 days was observed after 5[1/2] days. The relatively long latent period in the cow compared with that in sheep (about $2\frac{1}{2}$ days) may be attributable to the intermittent nature of the administration because parturition can be induced in as short a time at 3 days by continuous fetal infusion of dexamethasone (Fairclough *et al.*, 1981). Fairclough *et al.* (1981) described a dose relationship to latent period which was 12, 9 and 3 days respectively for daily infusion rates of dexamethasone of 0.1, 1.0 or 10.0 mg. The lowest dose was associated with a 10-fold increase in cortisol concentration during the 3 days before calving. These observations are consistent with the existence of negative feedback 6 weeks before term in the bovine fetus and also with a maturational effect of glucocorticoid on the hypothalamus–pituitary whereby the threshold of negative-feedback is progressively raised. A dose-related response in fetal oestrogen concentration also was found: with <2.2 mg of dexamethasone per day, no change in conjugated oestrogen concentration occurred, but at a dose of 10 mg/day fetal oestrogen values rose sharply before the rise in maternal oestrogen concentrations. Fairclough *et al.* (1976) postulated that fetal corticosteroids not only stimulate placental production of oestrogen but also influence the relative amounts of oestrogen secreted into the fetal and maternal circulations.

Maternally administered corticosteroids for inducing parturition have been extensively studied because of their commercial utility in inducing calving in dairy cattle that would

otherwise calve late in the milking season. In general, the patterns of concentrations of P_4, oestrogen and PGs in the maternal circulation after corticosteroid administration closely resemble those at normal term. However, the time course of the changes is strongly dependent on the gestational age, the latency to delivery ranging from 13 days at 240 days (Hunter *et al.*, 1974) to as short as 36 h at 272 days (B. Hoffman *et al.*, 1973).

Little work has been done in the past 15 years with chronically cannulated fetal preparations (probably because of the cost of experimental animals) and knowledge of the hypothalamo-pituitary–adrenal axis in fetal calves does not match that in fetal sheep. Although it is likely that many of the observations made in fetal sheep can be extrapolated to the bovine fetus, there is a need to apply recently developed techniques to a relatively few well-planned experiments to confirm the validity of extrapolation.

9.7.2 PROGESTERONE

The changes in P_4 concentrations in maternal peripheral plasma follow a well-defined pattern on which there is general agreement (R.V. Short, 1958; Gomes *et al.*, 1962; G.S. Pope *et al.*, 1969; Donaldson *et al.*, 1970; Edqvist *et al.*, 1970; Stabenfeldt *et al.*, 1970; Robertson, 1972; Schrams *et al.*, 1972; R.E. Short *et al.*, 1989). During the last 28 days of pregnancy, concentrations remain steady in the range 5–15 ng/ml until about 7 days before parturition, when they decline steadily to 3 ng/ml at 48 h before parturition. This fall coincides temporally with rising concentrations of cortisol in the fetus (Hunter *et al.*, 1977). During the final 48 h, P_4 values fall rapidly to <1 ng/ml. The maternal administration of dexamethasone in doses of 10 or 20 mg is associated with a sharp fall in P_4 concentration to about 1 ng/ml (Edqvist *et al.*, 1972) immediately before premature parturition.

The source of P_4 in bovine pregnancy has been controversial. The placenta can synthesize P_4, as demonstrated by incubations of homogenized tissue (Ainsworth and Ryan, 1967) or dispersed heterogeneous populations in culture (Shemesh *et al.*, 1984b), but the amounts are small compared with those released by placental tissue in other species. The cell type responsible for P_4 is the BNC (Reimers *et al.*, 1985) which, although of fetal origin, migrates into the maternal epithelium (Wooding and Wathes, 1980). However, there is no clear evidence that the placenta secretes P_4. Indeed, the concentration of P_4 in uterine venous plasma is lower than in peripheral venous or arterial blood (Comline *et al.*, 1974; Fairclough *et al.*, 1975), although S.M. Pimentel *et al.* (1986) found higher concentrations of P_4 in uterine venous blood than in arterial blood at 250 days and no difference at 270 days. Early ovariectomy experiments attempting to resolve the question of the relative roles of the placenta and CL suggested that the placenta might secrete P_4 in substantial amounts in late pregnancy because bilateral ovariectomy caused abortion only when performed before 200 days. However, when ovariectomy was performed after 200 days (Estergreen *et al.*, 1967) there was a fall in the concentration of P_4 to >10% of preoperative values (Edqvist *et al.*, 1973a), confirmed by Wendorf *et al.* (1983). These findings undoubtably show that the CL is the major source of P_4 in bovine pregnancy but leave unanswered the question of the source of the 10–20% of P_4 remaining in the plasma after bilateral ovariectomy. Fairclough *et al.* (1975) postulated that the maternal adrenal might be the source because Balfour *et al.* (1957) reported that the concentration of P_4 concentration in adrenal venous blood in pregnant cows at 210 and 240 days of gestation was 10–100 times greater than in adrenal arterial blood. Wendorf *et al.* (1983) compared the effects of bilateral ovariectomy, bilateral maternal adrenalectomy and a combination of the two procedures performed at

215 days: the duration of pregnancy in adrenalectomized animals compared with intact controls was unaffected, whereas ovariectomy was associated with delivery 8 days earlier than in controls: ovariectomized/adrenalectomized animals aborted within 4 days of operation. Plasma P_4 concentrations fell slightly after adrenalectomy, to 20% after ovariectomy and to undetectable levels after the combined operation. Wendorf *et al.* (1983) therefore concluded that, at least after 215 days of gestation, the placenta does not secrete P_4 and that the CL is the major source, and that the adrenal secretes sufficient P_4 to maintain pregnancy until rising oestrogen concentrations near term cause a marked shift in E/P_4 ratio. Nevertheless, the results of S.M. Pimentel *et al.* (1986) leave open the possibility that the placenta secretes small amounts of P_4 at 250 days.

The prepartum fall in P_4 appears to be obligatory for the initiation of parturition at term since administration of P_4 or MPA can prolong pregnancy by 7–10 days (L.E. McDonald *et al.*, 1953; Nellor, 1963). However, the prolongation is unpredictable and fetal death is not uncommon. McDonald and Hays (1958) treated cows with P_4-in-oil starting 33 days before term with a regimen that was able to prevent abortion after luteectomy, but observed no prolonged pregnancies. However, the dose of P_4 was probably insufficient to prevent a substantial fall in plasma P_4 at term. In other studies using similar doses of P_4, pregnancy was either not prolonged (Holm, 1967) or prolongation occurred in some but not all animals (Kiesel and Autrey, 1961). Fairclough *et al.* (1984) examined the effect of progestagens on premature parturition induced by intrafetal infusion of dexamethasone: the cows were infused intravenously with P_4 or megestrol acetate. Treatment with P_4 prevented the fall in P_4 concentrations in untreated cows. Parturition was prevented in all treated animals although concentrations of $PGF_{2\alpha}$ in utero-ovarian venous blood increased as in untreated animals. Delivery occurred within 2 days of stopping treatment with progestagen.

9.7.3 OESTROGENS

The concentration of unconjugated oestrone in plasma exceeds that of oestradiol-17β by 3–5 times, but the ratio is fairly constant throughout the second half of pregnancy. Oestrone concentrations are >100 pg/ml from 140 days of gestation until 260 days, when levels began to rise steadily to reach a peak at term of about 2000 pg/ml oestrone and about 200 pg/ml of oestradiol-17β (Edqvist *et al.*, 1973b). In general, these changes have been confirmed by others using radioimmunoassays (Robertson and King, 1974; B. Hoffmann *et al.*, 1977; Hunter *et al.*, 1977), but opinions differ as to whether a fall in concentration occurs during the 24 h before parturition. Peterson *et al.* (1975) and Hunter *et al.* (1977) reported well-defined peaks of both oestrone and oestradiol-17β beginning 7–10 days before term and reaching maximal values about 3 days before term before falling sharply during the 24 h before parturition (Figure 9.13).

There was no increase in fetal oestrogens accompanying the peak in maternal plasma. Maternal plasma also contains oestradiol-17β but in much lower concentrations than in fetal plasma, where it is the dominant oestrogen. Unconjugated oestrogens form only 1–10% of total oestrogens (B. Hoffmann *et al.*, 1977).

The source of oestrogen in the maternal circulation is probably the placenta; neither fetectomy (B. Hoffmann *et al.*, 1979) nor bilateral ovariectomy and bilateral adrenalectomy (Wendorf *et al.*, 1983) causes a fall in oestrogen values. The concentration of oestrogen in utero-ovarian venous blood is higher than that in peripheral blood (Robertson, 1972; Peterson *et al.*, 1975; G. Evans and Wagner, 1981). The nature and site of synthesis of oestrogen precursors remain uncertain, but the experiments described above make it

likely that they are derived largely, if not entirely, from C_{21} steroids synthesized in the placenta. Whether the precursors are Δ_4 or Δ_5 steroids derived from P_4 or P_5 respectively is also uncertain. Fetal placental minces and enriched preparations of BNCs convert P_5 primarily to P_4 in late pregnancy, but the product becomes predominantly oestrogens prepartum (Gross and Williams, 1988). The rate of conversion of P_5 to androstenedione

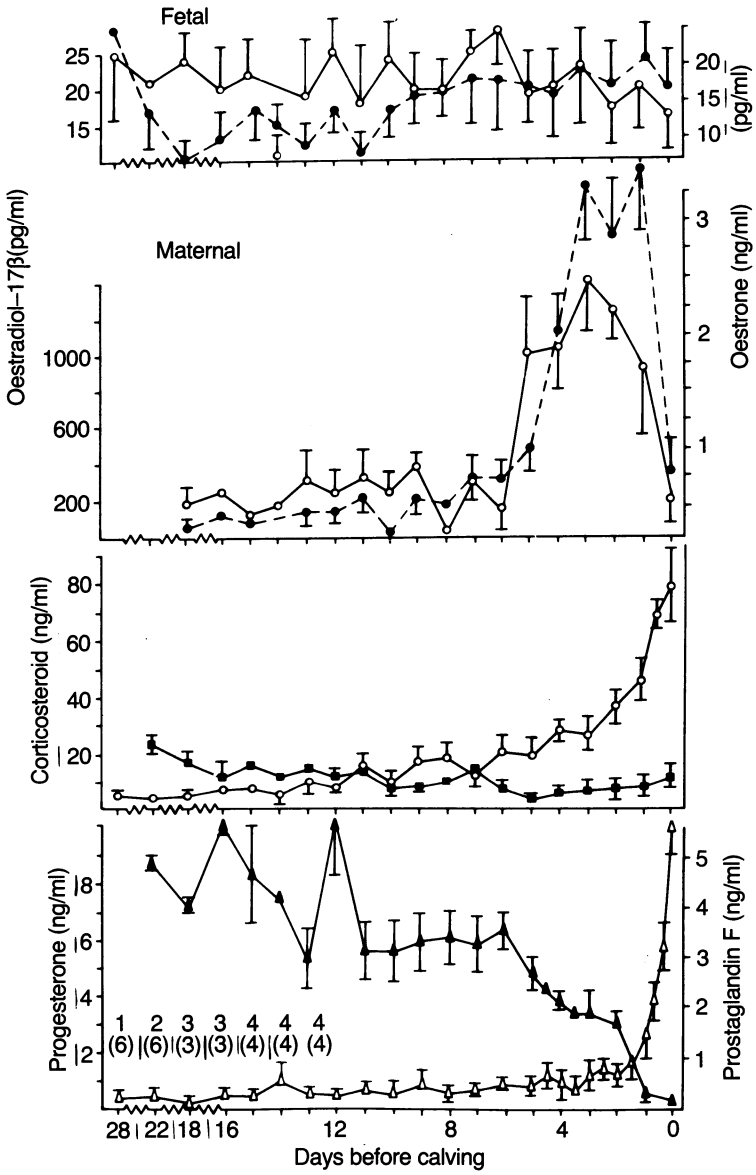

Figure 9.13 Plasma concentrations of fetal and utero-ovarian oestrone (●) and oestradiol-17β (○), fetal (○) and maternal (■) jugular corticosteroids, maternal jugular progesterone (▲) and utero-ovarian $PGF_{2\alpha}$ (△) preceding parturition in the cow. (Reproduced with permission from Fairclough *et al.*, 1976; 1984.)

and oestrogen is increased by exposure to dexamethasone. These findings *in vitro* are consistent with the observations *in vivo* that treatment of the pregnant cow or the fetus with glucocorticoids is associated with a marked peak in the concentration of plasma oestrogens (Fairclough *et al.*, 1981) and suggest that the prepartum rise in oestrogens is the result of activation by fetal cortisol of 17α-hydroxylase and C17,20-lyase, as it is in sheep. This is supported by the demonstration that exposure of placental tissue *in vitro* to glucocorticoids enhances the activity of 17α-hydroxylase (Flint *et al.*, 1979) and by the observation that maternal oestrogen concentrations increase in corticosteroid-treated, ovariectomized cows despite suppressed maternal and fetal adrenal activity (B. Hoffman *et al.*, 1977). The question of the relative importance of the Δ_4 and the Δ_5-pathways was investigated by Hoedemaker *et al.* (1990), who incubated fetal villi with various tritiated steroids. They confirmed a dose-dependent, stimulatory action of cortisol on the conversion of androstenedione and P_5 to oestrogen and found that androstenedione, P_5 and 17α-OH-P_4 were the preferred substrates. The relative conversion rates of androstenedione and DHEA to oestrogen were not investigated.

9.7.4 PROSTAGLANDINS

The concentration of $PGF_{2\alpha}$ in utero-ovarian venous plasma remains low (1 ng/ml) throughout the last month of pregnancy until 36–48 h before parturition. A gradual rise then occurs until 24 h prepartum, when the concentration rises sharply to reach 5–7 ng/ml (Fairclough *et al.*, 1975; Hunter *et al.*, 1977; Gimenez *et al.*, 1983) (Figure 9.13). The concentration of PGFM in peripheral blood follows a similar pattern (Edqvist *et al.*, 1978). Although there is no correlation of PGF or PGFM concentrations with the slow decline in P_4 concentrations during the last week of pregnancy, the final sharp fall in P_4 values during the final 24 h prepartum coincides in onset with the marked increase in PGF_2 in utero-ovarian plasma and of PGFM in peripheral plasma, suggesting a luteolytic role for $PGF_{2\alpha}$. Luteectomy on day 250 after infusion of oestrone for 4 days fails to stimulate secretion of $PGF_{2\alpha}$, indicating that the fall in P_4 production in normal cows is more likely to be a consequence of the sharp increase in $PGF_{2\alpha}$ than its cause (C.A. Pimentel *et al.*, 1986). Furthermore, the infrequent blood sampling regimens in all studies to date leave open the possibility that the final rise in $PGF_{2\alpha}$ is preceded by brief spikes of release of $PGF_{2\alpha}$, as occurs during luteolysis in the oestrous cycle.

The stimulus of $PGF_{2\alpha}$ secretion at term remains uncertain. Neither oestrone infusion at 246 days nor removal of the major source of P_4 by luteectomy at 250 days stimulates secretion (C.A. Pimentel *et al.*, 1986) but it is possible that an increase in E/P_4 ratio nearer term could be effective. Nevertheless, the increase in $PGF_{2\alpha}$ concentrations normally associated with the marked increase in E/P_4 ratio before parturition induced by intrafetal infusion of dexamethasone is not impaired by large doses of progestagen that block parturition (Fairclough *et al.*, 1984). In addition, increased concentrations of oestrogen in maternal blood are not obligatory for release of $PGF_{2\alpha}$ since intrafetal infusion of low doses of dexamethasone are effective in inducing release of $PGF_{2\alpha}$ and parturition although oestrogen levels are unchanged (Fairclough *et al.*, 1981). However, plasma oestrogen concentrations may be an insensitive indicator of concentrations within the endometrium close to the site of synthesis in the placenta.

An endometrial inhibitor of synthesis may also contribute to the regulation of PG release. Shemesh *et al.* (1984a) described an endogenous, heat-labile inhibitor extracted from maternal caruncular tissue of placentomes. The activity was present in extracts from 120–0 day placentomes but was not de-

tectable after 260 days. Co-cultures of 120-day tissue (but not term tissue) with fetal cotyledonary cells inhibited PG synthesis. Nothing is known of the factors responsible for the disappearance of inhibitory activity in late gestation. On the whole, while the evidence that release of $PGF_{2\alpha}$ is determined mainly by the E/P_4 ratio is inconclusive, it seems to be the likeliest mechanism. Studies with inhibitors of P_4 synthesis and P_4 receptors such as epostane and mifepristone respectively are needed to resolve the issue.

The source of PGs released into the uteroovarian vein is thought to be mainly the placenta. Synthesis of labelled PGs from tritiated arachidonic acid was greater in the fetal villus than the maternal crypt component of late gestation placentomes and increased prepartum in both components, the predominant product being PGE_2 from fetal tissue (Gross *et al.*, 1987). The latter observation is not consistent with the concentrations of PGE_2 in fetal blood reported by Gimenez *et al.* (1983): blood samples were obtained acutely from 80 to 260 days of pregnancy and the concentration of PGE_2 in the umbilical artery and vein was less than half that of $PGF_{2\alpha}$ or PGFM. Whether PGE_2 increases prepartum in the fetal calf circulation as it does in the sheep fetus is unknown. Highly enriched suspensions of BNCs produce PGE_2 and, to a lesser extent, PGI_2 (Reimers *et al.*, 1985), but the production of PGF_2 was not measured. Dispersed fetal binucleate cells, rather than fetal principal cells, were the major site of synthesis of PGs, and PGE_2 predominated (Gross and Williams, 1988).

The above studies do not appear to have clarified the source of the large quantity of $PGF_{2\alpha}$ released into the maternal circulation immediately prepartum. By analogy with other species, it is most likely to be maternal tissues, but incubation of minces of maternal caruncles are not confirmatory; the possibility that intercaruncular endometrium is more active in production of $PGF_{2\alpha}$ has not been explored.

9.7.5 OXYTOCIN

The concentration of oxytocin in peripheral plasma in late pregnancy is low (1–3 pg/ml), and no increase is found until the forelegs of the calf distend the vagina late in the second stage of labour (Landgraf *et al.*, 1983), confirming the early observations made with bioassays (Fitzpatrick and Walmsley, 1965). Maximal values of 60–116 pg/ml are reached at the time of delivery. Intravenous or intramuscular injections of pig relaxin near term stimulate oxytocin release within 30 min (Musah *et al.*, 1989), but the significance of this observation is unclear. The concentration of oxytocin receptors and the interrelationships of oxytocin and PGs have not been studied for cattle.

9.7.6 RELAXIN

Relaxin has been isolated from CL late pregnant cows (M.J. Fields *et al.*, 1980), but the concentration in the circulation has not been described. When pig relaxin is administered into the cervical canal in late pregnancy there is marked cervical dilatation, relaxation of the pelvic ligaments and shortening of the duration of pregnancy by about 2 days (Perez-grovas and Anderson, 1982; Musah *et al.*, 1986). There was also a sharp fall in P_4 values and a rise in oestrogen levels within 24 h of treatment, but the reasons are unknown. Intramuscular treatment with relaxin is ineffective (Caldwell *et al.*, 1990).

9.7.7 SUMMARY

The bovine placenta does not secrete P_4 in significant amounts and the source of circulating P_4 is mainly the CL, but the maternal adrenal may secrete sufficient P_4 to maintain pregnancy after ovariectomy. Parturition is initiated by luteolysis, probably induced by $PGF_{2\alpha}$ from a uterine source that remains undefined. The release of $PGF_{2\alpha}$ is stimulated by a sharp prepartum increase in concen-

trations of fetal cortisol, but the mechanism is uncertain since neither increased oestrogen levels nor reduced P_4 concentrations (or both) stimulate release. The roles of oxytocin and relaxin have not been elucidated. In many respects, the mechanism of initiation of parturition in cattle resembles that in goats.

9.8 HORSE

Information relating to parturition in the horse is insufficient to propose a coherent hypothesis for the mechanism of initiation of parturition. Available evidence makes it clear, however, that the horse differs markedly from ruminants and also differs in many respects from primates.

9.8.1 ROLE OF THE FETUS

The classical demonstration that the duration of pregnancy is determined by the genotype of the fetus was cross-breeding experiments in equids. The duration of pregnancy in the horse (stallion × mare) is 340 days and of the donkey (jack × jenny) is 365 days; the stallion × jenny cross is 350 days and the jack × mare cross is 355 days. However, these experiments cannot be interpreted as proving that the fetus itself plays the major part since the genes that are expressed may lie not in the fetus but in the placenta and/or fetal membranes, as they may in human pregnancy. The technical difficulties of obtaining surgical access to the fetal horse and of maintaining chronic preparations has prevented critical experiments such as fetal hypophysectomy and adrenalectomy. Experiments of nature in which the fetus lacks hypothalamic–pituitary tissue or adrenal glands have not been reported. The plasma concentrations of cortisol in the fetal horse are unusual in showing no increase until 48 h before parturition and a marked increase in concentration only during parturition. Rossdale and Silver (1982) point out the wide range of gestation lengths that are compatible with mature foals when the onset of labour is spontaneous and yet the likelihood of delivery of an immature fetus when labour is induced with PG analogues within the term range. They suggest that 'readiness for birth' is linked to fetal adrenal activity and occurs close to the natural time of delivery. These observations and the infrequency of premature birth under natural conditions suggest that a signal, whether endocrine or paracrine, must pass from the fetus to the mother to indicate the foal's readiness for birth. Whether the signal is dependent on heightened activity of the fetal hypothalamic-pituitary–adrenal system is unknown, but the ability of dexamethasone administered to the mare to shorten pregnancy (Alm *et al.*, 1975) is consistent with the notion. However, labour does not start for several days after dexamethasone treatment is stopped, which makes it unlikely that the corticosteroid acts directly as it does in sheep and goats.

Corticosteroid treatment accelerates maturation of the fetal horse (G.C. Liggins, unpublished observations) and it is possible that the effect of dexamethasone on the timing of parturition is expressed by advancing fetal maturation. The observation of Alm *et al.* (1975) that P_4 treatment was as effective as dexamethasone in shortening pregnancy length is unexplained.

9.8.2 PROGESTERONE

Ovariectomy in pregnant mares up to 70 days of gestation causes abortion unless followed by P_4 treatment but is compatible with continuing pregnancy when performed at 140 days (Holtan *et al.*, 1979), indicating that the placenta is a major source of P_4 throughout the second half of pregnancy. The pattern of plasma concentrations differs from that in other species. Peripheral concentrations increase early in pregnancy, peak at 60–120 days and subsequently fall to low (Holtan *et al.*, 1975; Seamans *et al.*, 1979; Seren *et al.*,

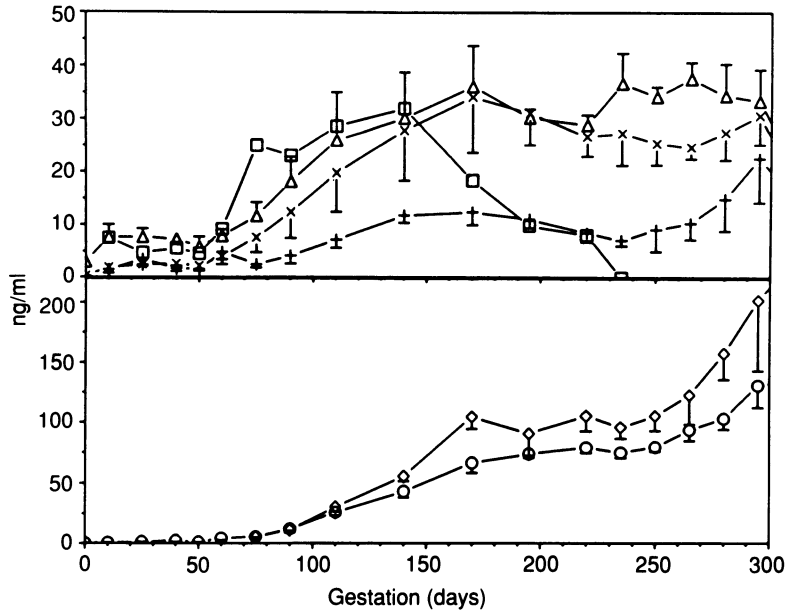

Figure 9.14 Plasma progestagens measured by GC–MS in pregnant pony mares. (□) = progesterone; (△) = 5α-pregnane-3,20-dione; (×) = 3β-hydroxy-5α-pregnan-20-one; (+) – 5α-pregnane-3β,20β-diol; (◇) = 20α-hydroxy-5α-pregnan-20-one; ( ) = 5α-pregnane-3β, 20α-diol. Means, ± SEM. (Reproduced with permission from Holtan *et al.*, 1991.)

1981; N.S. Pope *et al.*, 1987) or undetectable (Holtan *et al.*, 1991) values. Holtan *et al.* (1991), using highly specific gas chromatography–mass spectrometry (GS–MS), found no detectable P_4 after 240 days but substantial quantities of 5α-reduced metabolites, illustrating the problems associated with the use of less specific methods by earlier workers (Figure 9.14).

The predominant steroids are 20α-hydroxy-5α-pregnan-3-one (400–2100 ng/ml) and 5α-pregnane-3β,20α-diol (100–350 ng/ml). These first appear in the plasma between 30 and 60 days and increase gradually until about 30 days prepartum, when concentrations rise more rapidly until 2–3 days prepartum, when a slight fall occurs. Four other mono and dihydroxy-5α-pregnanes identified by Holtan *et al.* (1991) are present in relatively low concentrations (30–100 ng/ml). It is difficult to reconcile the results obtained by GC–MS with those of Seamans *et al.* (1979) using chromatography/radioimmunoassay (RIA), who observed marked falls prepartum in both 5α-dihydroprogesterone (from 130 to 10 ng/ml) and total hydroxy-5α-pregnanes (from 1400 to 100 ng/ml). Present evidence suggests that circulating P_4 is not required for the maintenance of pregnancy after 200 days but the role that 5α-pregnane metabolites might play remains uncertain until information about their biological activities becomes available.

Fowden and Silver (1987) investigated the effects of inhibiting P_4 synthesis by administering epostane, a 3β-HSD inhibitor, for 9 days at 292 days of pregnancy. Although plasma P_4 concentrations (measured by RIA) fell rapidly and remained depressed throughout treatment, none of the mares delivered prematurely. The results of similar experiments with the P_4 receptor blocker, mifepristone, may help resolve the question of whether progesterone or its metabolites have

a significant place amongst the hormones maintaining pregnancy and controlling parturition.

9.8.3 OESTROGENS

The equine placenta lacks 17α-hydroxylase and requires androgen as substrate for synthesis of oestradiol-17β and oestrone (Ainsworth and Ryan, 1969). The fetal gonad rather than the fetal zone of the adrenal is the source of DHEAS metabolized by the equine placenta (Raeside *et al.*, 1979). Regression of the fetal gonads in late pregnancy is reflected in falling concentrations of plasma oestrogens including biologically inert equilin and equilenin in the mare. This suggests that the gonads are the source of substrate for oestrone and equilin, but the nature of the substrate for equilin is unknown because administration of labelled DHEA to the fetus yields labelled oestrone but not equilin (Pashen *et al.*, 1982). The concentration of oestradiol-17β in maternal plasma rises from very low values after 108 days until 153 days, when levels rise rapidly to peak values at 198 days then decline progressively after 279 days, but the interval from peak values to parturition varies greatly (Haluska and Currie, 1988). No significant changes in the concentration of oestradiol-17β occur near parturition.

Fetal gonadectomy results in an immediate and sustained fall in maternal plasma oestrogen concentrations, but P_4 values are unaffected. Dysmature foals are delivered at term (Pashen and Allen, 1979). Parturition can therefore occur normally despite the maintenance of a very high P_4–oestrogen ratio. The administration of large doses of stilboestrol to mares from 321 days to foaling has no effect on the length of gestation, although softening and dilatation of the cervix occurs soon after the start of treatment (Alm *et al.*, 1975). These observations indicate that oestrogen is unlikely to play a significant part in initiating parturition, even permissively.

9.8.4 OXYTOCIN

The myometrium of the pregnant horse is very sensitive to oxytocin near term and parturition can be induced rapidly by a single dose of oxytocin (Hillman, 1975; Rossdale *et al.*, 1984), which presumably reflects a high concentration of oxytocin receptors. It is likely that the ability of oxytocin to induce rapid delivery is dependent on prior changes in the cervix that render it distensible, but this has not been studied. Nothing is known of the sensitivity to oxytocin earlier in pregnancy or of the factors that influence the formation of oxytocin receptors. The patterns of P_4 and oestrogen concentrations in late pregnancy do not encourage the view that the sensitivity to oxytocin is determined by these steroids.

The concentration of oxytocin in the peripheral circulation remains at very low values throughout pregnancy (Haluska and Currie, 1988) until the expulsive stage of labour, when peaks of variable magnitude are found (Allen *et al.*, 1973; Haluska and Currie, 1988).

Mares have an usually high degree of maternal control over the expulsive phase of labour, suggesting that the high sensitivity to oxytocin coupled with the low circulating concentrations provides a means for the maternal control of parturition by inhibiting release of oxytocin through neural mechanisms (Haluska and Currie, 1988). The administration of oxytocin is followed immediately by the release of $PGF_{2\alpha}$ (Barnes *et al.*, 1978; Rossdale *et al.*, 1979), although manual dilatation of the cervix is not, indicating that a cervical reflex stimulating oxytocin release is absent in the horse.

9.8.5 RELAXIN

Relaxin in peripheral plasma assayed by heterologous RIA shows no change prepartum or until the expulsive stage of labour, when there is a sharp increase (Stewart *et al.*,

1982), probably due to a response to oxytocin because it can be reproduced by an intravenous bolus of oxytocin, but mediation by the release of $PGF_{2\alpha}$ cannot be excluded. The source of relaxin is likely to be the placenta; relaxin concentrations in the blood persist after delivery while the placenta is retained but fall after expulsion, and release of relaxin can be stimulated by oxytocin while the placenta is retained but not after expulsion (Stewart *et al.*, 1982).

The function of relaxin in equine parturition has not been studied and there is no direct evidence that it is involved in the connective tissue changes in the cervix and pelvic ligaments associated with parturition or with myometrial contractility. The nonpregnant cervix is notable for its unusual distensibility, having longitudinal folds rather than annular rings, as in other farm species, and readily permits the passage of large instruments (Ginther, 1979). This suggests that relaxin may be relatively unimportant in relation to prepartum cervical function.

However, studies of EMG activity in the myometrium in two pony mares led Haluska *et al.* (1987a) to propose that the unusual pattern of myometrial activity in equine parturition is attributable to the effects of relaxin. During the week before parturition, uterine activity has a diurnal rhythm with infrequent contractions during the day and increased activity at night. On the day before parturition, increased activity is present during the day and becomes persistently increased during the early daylight hours of the day of parturition. However, a phase of myometrial quiescence lasting 2–4 h separates labour from rupture of the membranes and the expulsive phase of labour, a pattern that appears to be unique to the mare. The quiescent phase precedes the sharp increase in relaxin concentrations during the expulsive phase. Immediately after delivery, myometrial quiescence returns and persists until the placenta is expelled.

When the photoperiod was reversed for 40 days before expected term, 58% and 16% of mares delivered at 08:30–16:30 h and 22:30–08:30 h, respectively, compared with 13% and 80% of control mares at these times (Bosc *et al.*, 1988).

9.8.6 EICOSANOIDS

Daily intramuscular injections of 12 mg of $PGF_{2\alpha}$ in mares in late pregnancy does not induce labour (Alm *et al.*, 1975), possibly because of inadequate dosage. The synthetic analogue, fluprostenol, is very effective, stimulating labour and delivery within 90 min in most animals (Rossdale *et al.*, 1979). The administration of fluprostenol is associated with an almost immediate increase in plasma concentrations of PGFM, which rise to very high levels at delivery if foaling occurs within 90 min. In mares foaling after >90 min, PGFM values fall rapidly at the time of delivery, suggesting that the release of PGFM is not directly related to uterine activity or distension of the birth canal. Measurements of oxytocin have not been made, and it is not known whether fluprostenol stimulates release of oxytocin, which could mediate the effect of fluprostenol on release of $PGF_{2\alpha}$. Rossdale *et al.* (1979) considered this to be unlikely because of the variable time (up to 60 min) from injection of fluprostenol to the release of $PGF_{2\alpha}$. These experiments cannot be interpreted as unequivocal evidence that $PGF_{2\alpha}$ acts on the myometrium or has an obligatory role in parturition. The effects of cyclo-oxygenase inhibitors such as indomethacin on the time of parturition in horses have not been reported.

The concentration of PGFM in maternal plasma is low until after 200 days, when a progressive rise occurs to a plateau about 15 days before term, which is maintained until parturition (Barnes *et al.*, 1978; Stewart *et al.*, 1984; Haluska and Currie, 1988) (Figure 9.15).

PGFM concentrations rise sharply in labour but not until a few minutes before rupture of

the membranes and at least 2 min after an eightfold increase in the concentration of oxytocin has occurred (Haluska and Currie, 1988). The release of $PGF_{2\alpha}$ may therefore be a consequence of the rise in oxytocin concentration since administration of oxytocin is followed within a brief interval by a marked increase in PGFM values. The existence of significant arteriovenous differences in PGFM across the uterine circulation in late pregnancy strongly suggests uterine tissues as the source of PGFM, but the precise tissue is unknown (Silver *et al.*, 1979a).

Concentrations of PGE_2 and $PGF_{2\alpha}$ increase in fetal plasma and allantoic fluid 2–6 days before parturition. The source of the PGs and the cause of the increase are uncertain, although they may be related to a fall in P_4 concentration in fetal plasma that occurs over the same period (Barnes *et al.*, 1978).

An unusual feature of PG metabolism in the horse is the marked increase in the output of PGFM from the uterus during fasting for 20–30 h (Silver and Fowden, 1982). The increase in plasma concentration of PGFM correlates with the fall in plasma glucose and the rise in free fatty acids. The levels of PGFM fall promptly after refeeding or glucose infusion, reaching basal levels within 1–3 h. Despite maintenance of normal nutrition after fasting there is a strong tendency to premature parturition within 1 week. These observations indicate that a mechanism might exist whereby the fetus or placenta can recognize nutritional deprivation and respond by setting in motion the means to escape from an unfavourable intrauterine environment. However, it is surprising that the response should occur after such a brief period of undernutrition.

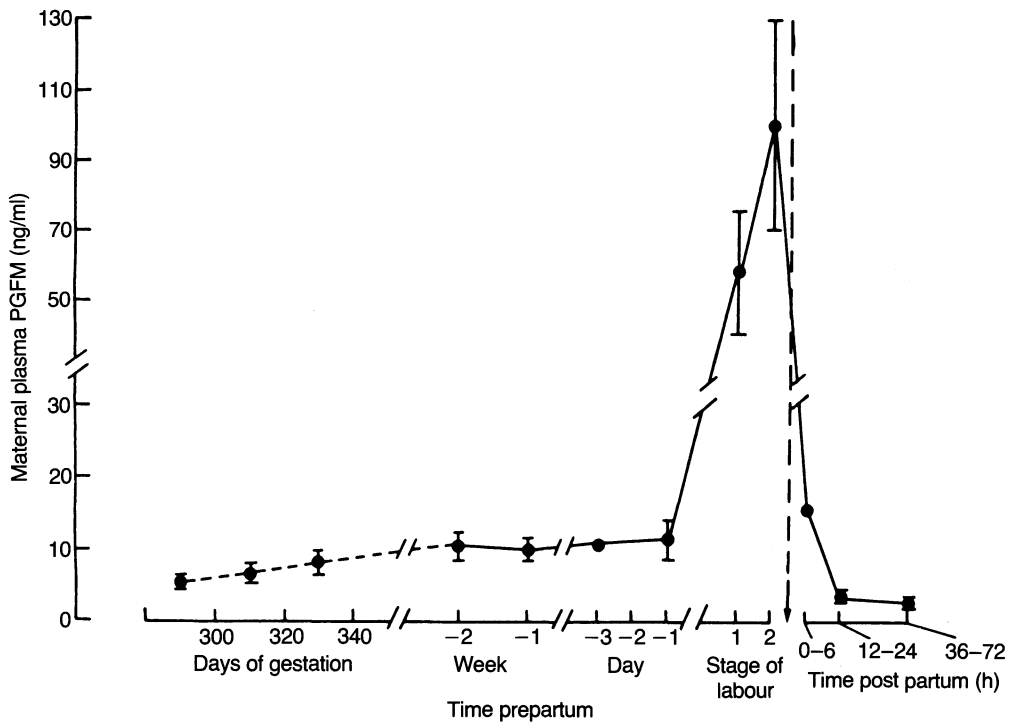

Figure 9.15 Concentration of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) in peripheral plasma through pregnancy and parturition in mares. (Reproduced with permission from Barnes *et al.*, 1978.)

9.8.7 SUMMARY

The horse shows a remarkable sensitivity of the myometrium to oxytocin and the speed with which delivery can be induced after a single intravenous injection of oxytocin. The late onset of raised oxytocin concentrations only minutes before the expulsive phase of labour is difficult to reconcile with the increased uterine activity present for several hours before delivery unless the myometrial sensitivity to oxytocin is increasing during this period. Studies of oxytocin receptor concentrations are needed.

Convincing evidence that oestrogen, P_4 or prostanoids play a significant part in the initiation of parturition in the horse is lacking (Thorburn, 1993). The oestrogen–P_4 ratio is unimportant. The effects of oxytocin antagonists on PG release and of cyclo-oxygenase inhibitors on oxytocin release and parturition need to be investigated. At present, the most likely sequence is that oxytocin receptor concentrations rise progressively in the final days of pregnancy, perhaps in response to release of minimal quantities of an eicosanoid, allowing the myometrium to respond to the low levels of circulating oxytocin. Eventually a mechanical stimulus triggers a massive release of oxytocin, which in turn stimulates the synthesis of $PGF_{2\alpha}$; rapid expulsion of the fetus is then assured by the combined effects of the two hormones. However, this hypothesis begs the question of how the fetus communicates its readiness for birth to the parturitional mechanism. This precocial species has a remarkably wide range of pregnancy lengths yet fetal maturation compatible with extrauterine survival seems to be attained only near the time of birth.

9.9 RAT

9.9.1 INTRODUCTION

Pregnancy in the rat is maintained by P_4 secreted by the CL and parturition is preceded by a fall in circulating concentrations of P_4. Although the pregnant rat is the subject of more reports than any species other than man, the factors determining the fall in the secretion rate of P_4 are unknown. The onset of parturition in the rat and probably other murids has several features that are unique to the species and make it unsuitable as a model with relevance to parturition in primates.

9.9.2 ROLE OF THE FETUS

Implantation of fertilized ova in lactating rats does not take place at the normal time but is delayed for several days. Administration of P_4 or oestrogen shortens the delay (Yoshinaga and Hosi, 1958). This phenomenon was exploited by Yoshinaga (1961) to study the effect of the fetus on the time of parturition by inducing early implantation of some of the blastocysts with local injections of oestradiol, thus creating pregnancies containing fetuses of two different ages. Delivery of all the fetuses takes place at one time in this preparation and the time depends on the ratio of old fetuses to young fetuses. When the ratio is more than 0.3, parturition occurs at normal term (23–24 days), but if the ratio is less than 0.3 parturition is delayed until 28 days, when the older fetuses are born dead and the younger fetuses are born alive. Decapitation of the older fetuses when the ratio is more than 0.3 results in parturition at 28 days. The results were interpreted as evidence that the fetal brain or pituitary secretes a hormone that initiates parturition. However, the fetus could be secreting a hormone that maintains placental production of a luteotrophin.

No evidence that the fetal brain or pituitary plays a part in initiating labour was found by Swaab *et al.* (1977), who removed the brain and pituitary by aspiration at 19 days of pregnancy. Brain-aspirated and sham-operated animals did not differ in the length of pregnancy, although both groups had longer pregnancies than unoperated controls. Brain-

aspirated animals had more protracted labours than sham-operated controls, suggesting that the fetus may contribute to the course of labour although not to its initiation. Schriefer *et al.* (1982) proposed that parturition is triggered by oxytocin release from the fetus, but Higuchi *et al.* (1985a) found no evidence of a fall in pituitary content of oxytocin or increased plasma oxytocin in newborn animals compared with fetuses near term.

9.9.3 PROGESTERONE

The major source of P_4 in pregnancy is the CL and ovariectomy at any stage of pregnancy is followed by abortion in 12–24 h (Csapo and Wiest, 1969; Clabaut *et al.*, 1988) which can be prevented by the administration of P_4. Abortion is also induced by treatment with P_4 antiserum (Csapo *et al.*, 1975) and by the inhibitors of P_4 synthesis, azastene (Creange *et al.*, 1978) or isoxazole (Carnathan *et al.*, 1981) and by mifepristone (Bosc *et al.*, 1987). The concentration of P_4 in the uterine vein is higher than that in arterial blood, suggesting placental secretion, but very much lower than that in ovarian venous effluent (Sanyal, 1978). Homogenates of mid-pregnancy placentas produce P_4 during incubation but the production rate in placentas obtained in later pregnancy is low (Matt and Macdonald, 1984). The abortifacient effect of ovariectomy leaves no doubt that the placenta secretes insufficient P_4 to maintain pregnancy. The concentration of circulating P_4 falls sharply beginning about 36 h before the onset of parturition (Bartholomeusz *et al.*, 1976; Clabaut *et al.*, 1988). In animals of other species, the mechanism causing reduced P_4 secretion is luteolysis, but the fall in P_4 secretion by the rat CL is due, at least in part, to a sharp 72-fold increase in ovarian metabolism of P_4 to 20α-hydroxypregn-4-en-3-one (20α-P_4) (Weist, 1970). The prepartum fall in P_4 concentrations is associated with an equally large rise in 20α-P_4 levels due to a marked increase in the activity of ovarian 20α-hydroxysteroid dehydrogenase (Weist *et al.*, 1968).

Induction of a new crop of CL after 12 days of pregnancy by injections of pregnant mare serum gonadotrophin followed by LH prolongs pregnancy to 25–27 days and usually results in death of the mothers or fetuses (Takahashi *et al.*, 1979). Plasma P_4 concentrations are maintained after term and the normal increase in 20α-P_4 is greatly attenuated. These results were interpreted as evidence that the secretion of placental luteotrophin continues beyond term and that the new CL remain responsive to placental luteotrophin, the difference being attributable to low levels of LH at the time when the new CL were formed.

The prepartum fall in P_4 levels is accompanied by an equally sharp fall in the concentrations of myometrial nuclear P_4 receptors and a sharp rise in nuclear oestrogen receptors (Saito *et al.*, 1985). This pattern of changes is consistent with P_4 promoting the formation of its own receptors and inhibiting formation of oestrogen receptors, thus amplifying the effects of lowered P_4 concentrations on target tissues.

Contractile activity in the rat uterus continues throughout pregnancy despite high concentrations of P_4 (Fuchs, 1969). Paradoxically, quiescence develops during the period of 24–36 h before parturition at a time when P_4 values are falling. Administration of P_4 12 h before term is followed by normal labour at the expected time (Bernard and Ruttner, 1985). These observations suggest that P_4 may lack a direct inhibitory action on the myometrial smooth muscle. Porter and Challis (1974) found no lessening of uterine activity post partum during intrauterine treatment with P_4 for 24 h. Because oestrogen inhibits uterine activity but promotes propagation and P_4 inhibits oestrogen receptor formation, Fuchs (1978) suggested that the observed actions of P_4 are best explained as anti-oestrogen effects. According to this hypothesis, falling concentrations of P_4 at

term are associated at first with uterine quiescence due to increased expression of oestrogen, but this gives way to labour contractions as gap junctions, oxytocin receptors and PG synthesis increase in response to oestrogen. *In vitro* studies of the effects of P_4 on longitudinal and circular muscle strips show that the acute effects are complex (Osa and Ogasawara, 1984). In late pregnancy, P_4 potentiates contractions of longitudinal muscle but inhibits those of circular muscle; post partum, the action on longitudinal muscle is biphasic and on circular muscle is inhibitory.

9.9.4 OESTROGEN

The rat placenta lacks aromatase activity and does not synthesize oestrogens (Matt and MacDonald, 1984). The evidence so far suggests that the luteotrophic action of oestradiol-17β in the rat is dependent on stimulation of intraluteal oestradiol production by LH and maintenance of oestradiol receptors by prolactin or a decidual luteotropin, probably rat placental lactogen, depending on the stage of pregnancy (Gibori *et al.*, 1978, 1981; Basuray *et al.*, 1983). The ovaries are the sole source of oestrogen in pregnancy, but luteal and non-luteal tissues have limited ability to synthesize oestrogen *in vitro* in the absence of added androgen substrate in the second half of pregnancy (Taya and Greenwald, 1981).

There is good evidence that the placenta is the major source of androgen for ovarian oestrogen synthesis and that androstenedione is the preferred substrate. Although activities of 17α-hydroxylase and 17,20-lyase are absent from the placenta in early pregnancy, activities of both enzymes increase steadily from 11 days and peak at 18 days' gestation (Warshaw *et al.*, 1986). The total capacity of the placenta for androgen production greatly exceeds that of the ovaries. Peripheral plasma concentrations of androstenedione and testosterone follow the same pattern as the activity of placental 17α-hydroxylase, rising from 12 days and peaking at 18 days (Jackson and Albrecht, 1985). The lack of effect on pregnancy length of aspirating the fetal brains in entire litters (which presumably prevents only prepartum increase in cortisol levels) does not support this possibility that placental 17α-hydroxylase is inducible by rising concentrations of fetal cortisol, as in other species, thereby providing the fetus with the means of controlling steroidogenesis. However, further studies of the regulation of placental 17α-hydroxylase are needed. Concentrations of oestrone and oestradiol-17β rise progressively from about 16 days of pregnancy and peak at 21 days before falling slightly before parturition starts (Labsethwar and Watson, 1975; Fuchs, 1978). Although circulating concentrations of oestrone are higher than those of oestradiol-17β, the reverse is true in the ovarian vein (Shaik, 1971). The secretion rate of the oestrogens follow a pattern similar to concentrations in plasma and reaches 100 ng of oestradiol-17β per 24 h.

Oestrogen has an essential role in pregnancy and parturition. After ovariectomy in late pregnancy, oestrogen administration maintains pregnancy and normal parturition occurs at term, whereas untreated animals have prolonged delivery with a high fetal mortality (Catala and Deis, 1973), probably because formation of oxytocin receptors and gap junctions is impaired by lack of oestrogen. Although oestrogen is luteotrophic in rats and delays luteolysis and parturition when given at 20 days at the start of luteolysis (Behrman *et al.*, 1971), parturition is advanced by administration at 21 days when luteolysis is established (Dukes *et al.*, 1974). Daily injections of oestradiol starting at 16 days induces premature delivery at 19 days (Mackenzie and Garfield, 1986). Oestrogen may therefore advance or delay delivery depending on the timing of treatment. The mechanism by which oestrogen stimulates premature delivery is uncertain but is likely

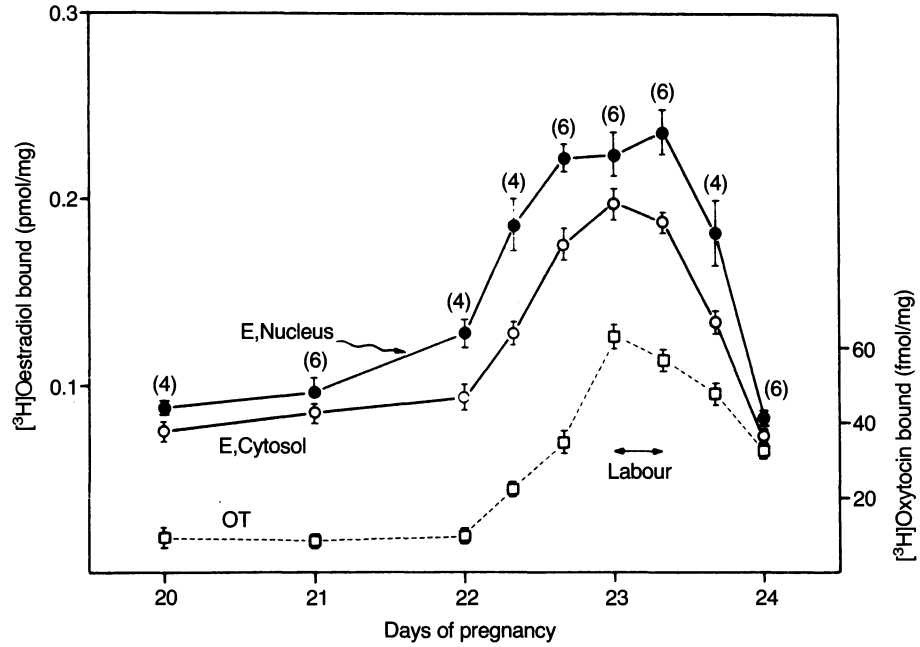

Figure 9.16 Concentrations of oxytocin receptors (□) and oestrogen receptors in the nuclear (●) and cytosolic (○) fraction of rat myometrium. (Reproduced with permission from Alexandrova and Soloff, 1980a.)

to be an indirect action since oestrogen inhibits uterine contractility *in vivo* and *in vitro* (Saldivar and Melton, 1966; Osa and Ogasawara, 1984). In ovariectomized puerperal rats, actinomycin D interferes with the inhibitory effects of oestradiol on spontaneous activity, suggesting that the mechanism involves the synthesis of protein (Downing and Porter, 1978).

9.9.5 OXYTOCIN

The myometrium is refractory to oxytocin throughout pregnancy until 6–8 h before delivery, when sensitivity increases markedly (Fuchs and Poblete, 1970). At that time, parturition is readily induced by infusion of oxytocin in very small amounts and the pattern of contractions mimics that during spontaneous delivery. Concentrations of oxytocin in maternal plasma are low until the first pup is delivered, when there is a modest rise (Higuchi *et al.*, 1985a,b). The rapid increase in sensitivity to oxytocin is attributable to a corresponding increase in the concentration of high-affinity oxytocin binding sites in the gravid and non-gravid uterine horns (Alexandrova and Soloff, 1980a,b). Attempts to establish increased oxytocin receptors as the major factor initiating parturition have not been successful. Passive immunization with an oxytocin antibody that impairs lactation is ineffective in preventing normal parturition at term (Schriefer *et al.*, 1982). Experiments with competitive antagonists of oxytocin are needed.

The concentration of oxytocin receptors correlates closely with that of nuclear oestradiol receptors, which increase as the concentration of P_4 falls prepartum (Alexandrova and Soloff, 1980b) (Figure 9.16).

The relationship to oestradiol is probably direct but it may be mediated indirectly, at least in part, by $PGF_{2\alpha}$ because adminis-

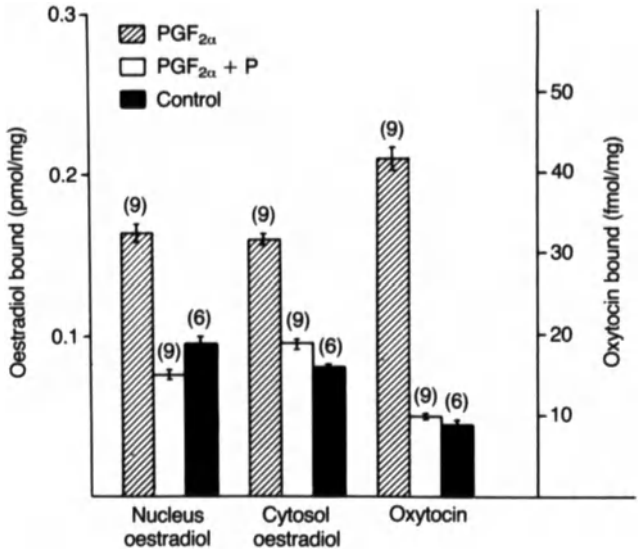

Figure 9.17 Effect of $PGF_{2\alpha}$ at day 18 on the concentration of oestradiol receptors in the nuclear and cytosol fraction and of oxytocin receptors in rat myometrium at labour on day 20. One group of $PGF_{2\alpha}$-treated animals was treated with progesterone. (Reproduced with permission from Alexandrova and Soloff, 1980c.)

tration of the cyclooxygenase inhibitor, naproxen, inhibits both the sensitivity to oxytocin and the increase in oxytocin receptors (W.Y. Chan *et al.*, 1988). However, in non-pregnant rats, treatment for 5 days with $PGF_{2\alpha}$ or indomethacin is without effect on oxytocin receptor concentrations (Engstrom *et al.*, 1988). The layers of the myometrium have different sensitivities to oxytocin: the longitudinal layer is as sensitive at 10 days of pregnancy as at term whereas the circular layer is refractory until 21 days (Crankshaw, 1987). A difference in the spontaneous contractile activity and response to oxytocin of the distal and proximal portions of the uterine horn correlates directly with differences in the concentrations of oxytocin receptors in the respective horns and inversely with the concentration of P_4 in the respective tissues (Gorodeski *et al.*, 1990). The administration of $PGF_{2\alpha}$ at 18 days induces delivery at 20 days accompanied by increased oxytocin and oestradiol receptors, both of which are inhibited by the administration of P_4 (Alexandrova and Soloff, 1980c) (Figure 9.17).

Whether the response is caused directly by $PGF_{2\alpha}$ or indirectly by inducing luteolysis remains uncertain. The administration of mifepristone causes fetal death about 12 h later. Oxytocin receptor concentrations rise sharply at this time but abortion is delayed for a further 12 h (Gorodeski *et al.*, 1990), suggesting that oxytocin may be insufficient to cause coordinated contractions and another factor, possibly PG, is essential for delivery.

Pelvic neurectomy impairs the release of oxytocin during parturition by interrupting the afferent pathways of a reflex stimulated by vaginal distension. Rats in which the increase in oxytocin is partly inhibited begin labour at term but delivery is slowed, whereas those showing no rise fail to deliver, but signs of labour such as stretch movements, vaginal bleeding and passage of mucus occur at term (Higuchi *et al.*, 1986).

These observations are consistent with increased oxytocin concentrations being obligatory for expulsion of the fetuses but not for the initiation of parturition.

9.9.6 RELAXIN

Relaxin in pregnancy arises from the ovary and disappears from the circulation after ovariectomy. Serum concentrations increase prepartum in two phases, the second of which occurs between 36 and 24 h before birth and coincides with the onset of functional luteolysis (Sherwood *et al.*, 1980). Concentrations fall during the final 24 h of pregnancy. Relaxin is required for normal delivery at term. Pregnant rats ovariectomized at 9 days and given maintenance P_4 (withdrawn at 21 days) and oestrogen (Downing and Sherwood, 1985a) or at 21 days and given oestrogen alone (Cheah and Sherwood, 1988) have prolonged pregnancies and durations of labour. Addition of relaxin to the treatment schedule restores pregnancy length and labour to normal. Treatment of intact rats with relaxin from 19 to 23 days prolongs pregnancy but not labour unless delivery occurs during treatment when labour is prolonged (Jones and Summerlee, 1986a). When delivery occurs after treatment is finished, labour is shortened. This may be attributable to a central effect of relaxin on oxytocin release since infusion of relaxin lowers circulating oxytocin concentrations (Jones and Summerlee, 1986a). The opioid antagonist, naloxone, prevents the fall in oxytocin values and the prolonged labour associated with relaxin treatment, suggesting that the effect of relaxin on oxytocin release is mediated by an opioid system (Jones and Summerlee, 1986b). However, naloxone treatment is ineffective in preventing delayed onset of labour in stressed rats although it shortens the interval between the birth of successive pups (Long *et al.*, 1985). Passive immunization with monoclonal antibodies specific for rat relaxin has no effect on the timing of luteolysis or initiation of parturition but prolongs labour (Guico-Lamm and Sherwood, 1988) due to failure of the cervix to undergo the normal prepartum increase in distensibility (Hwang and Sherwood, 1988).

The second phase of relaxin release that coincides with luteolysis can be induced prematurely by administration of $PGF_{2\alpha}$ (Gordon and Sherwood, 1983). Indomethacin protracts luteolysis, delays or prevents delivery and delays the increase in relaxin concentrations until indomethacin treatment is stopped.

In addition to effects on the cervix, relaxin inhibits myometrial activity. The frequency of contractions in ovariectomized animals maintained on oestrogen and P_4 is reduced by treatment with relaxin (Downing and Sherwood, 1985a). Relaxin inhibits spontaneous activity of circular muscle *in vitro* as well as activity induced by low concentrations of oxytocin and PGF but does not inhibit longitudinal muscle contractility (Downing and Sherwood, 1985b). Another potential route by which relaxin may influence myometrial activity is by its action in inhibiting myometrial synthesis of PGI_2 which stimulates myometrium *in vitro* (Gimeno *et al.*, 1983).

9.9.7 CATECHOLAMINES

Adrenaline and noradrenaline inhibit contractile activity of the longitudinal muscle from pregnant rats *in vitro*, whereas circular muscle is inhibited by adrenaline but stimulated by noradrenaline in low concentrations, particularly in late pregnancy (Chernaeva, 1984). The latter response probably reflects the concentration of α_1-adrenoreceptors, which increases sharply in the last 6 h of pregnancy (Legrand *et al.*, 1987) for reasons that are uncertain. The occupancy of α_1-adrenoceptors may make an important contribution to the overall excitability of the myometrium at the onset of labour because

chemical sympathectomy with 6-hydroxydopamine blocks parturition and markedly lowers the concentration of noradrenaline in both plasma and myometrium (Legrand *et al.*, 1987). Administration of a bolus of prazosin delays delivery for a period equal to the half-life of the drug. The relationship of α-adrenoceptors mediating these responses to the adrenergic nervous system is unclear in view of the progressive degeneration of the nerves during pregnancy.

9.9.8 EICOSANOIDS

Intravenous infusion of $PGF_{2\alpha}$ for 6 h at a rate of 20 g/h at 18 days or later induces irreversible luteolysis, as indicated by a fall in plasma P_4 concentration within 3 h and parturition 36–48 h later (Dukes *et al.*, 1974; Fuchs *et al.*, 1974). This suggests that release of $PGF_{2\alpha}$ at about 20 days of pregnancy is the normal luteolytic signal that initiates parturition, but there is little direct evidence to support this hypothesis. The anti-inflammatory agent, fenclozic acid, given at 20 days is ineffective in delaying labour (Dukes *et al.*, 1974). Treatment with indomethacin delays parturition by about 24 h (Aitken, 1971; Chantharaksri *et al.*, 1974; Fuchs *et al.*, 1976), which probably results from a reduced rate of decline of P_4 concentrations. Nevertheless, rising levels of 20α-P_4 show that luteolysis occurs despite indomethacin treatment. A delay of 24 h in the onset of parturition is associated also with passive immunization with anti-$PGF_{2\alpha}$ antibodies (Dunn *et al.*, 1973). Cavaille and Maltier (1978) demonstrated in unilaterally pregnant rats that removal of the ovary on the side of the gravid horn caused delayed parturition, whereas unilateral ovariectomy had no effect on pregnancy length in bilaterally pregnant animals. Their suggestion that the gravid horn generates a luteolytic factor that reaches the ovary by a direct route was supported by the observation that deliveries were delayed or incomplete in half the bilaterally pregnant animals in which unilateral ovariectomy was combined with ligation of the uterine artery and vein on the opposite side.

Although aspirin and indomethacin inhibit both spontaneous and oxytocin-induced contractions *in vitro*, indomethacin is ineffective in inhibiting contractions *in vivo* (Fuchs *et al.*, 1976). Furthermore, infusion of $PGF_{2\alpha}$ or PGE_2 within 24 h of term fails to induce delivery, whereas oxytocin is highly effective (Dukes *et al.*, 1964). These observations suggest that the rat differs from many other species in the relative refractoriness of the myometrium to PGs close to term. Progesterone enhances and oestrogen inhibits the myometrial response to PGs in ovariectomized rats (Fuchs, 1964); if this also occurs in the pregnant animal, the sharp prepartum fall in P_4–oestrogen ratio should inhibit the response to PGs.

In view of the doubt about the luteolytic role of endogenous $PGF_{2\alpha}$ and the refractoriness of the myometrium, the question arises as to the function of the rapidly increasing concentrations of prostanoids in the uterus near term (Gu and Rice, 1991). The withdrawal of P_4 in the presence of maintained oestrogen concentrations in ovariectomized pregnant rats maintained by steroid administration causes a specific increase in PGF concentration in uterine tissue and oestrogen alone stimulates PGF, PGE, TXB_2 and 6-keto-$PGF_{1\alpha}$ (L. Wilson and Lindsey, 1987), thus reproducing the marked increase in uterine PGs found after luteolysis at term in intact animals (L. Wilson and Freinkel, 1982). P_4 treatment blocks the stimulatory effects of oestrogen. Neither treatment increases the concentration of any of the prostanoids in uterine venous plasma (L. Wilson and Huang, 1984).

The major prostanoid produced by the myometrium is PGI_2, but experiments to determine its actions are inconclusive. Kawano and Mori (1988) observed that PGI_2 concentrations were highest where the uterine wall was most stretched and postulated

that PGI_2 maintained uterine relaxation. However, PGI_2 stimulates myometrium *in vitro*.

Although the prostanoids appear to have little direct oxytocic activity at term, they may play an important part in the mechanism causing the marked increase in concentration of oxytocin receptors that precede the onset of parturition (Alexandrova and Soloff, 1980c; W.Y. Chan *et al.*, 1988).

9.9.9 SUMMARY

The onset of parturition in rats is precipitated by functional luteolysis, which is unusual in that the fall in P_4 secretion is caused mainly by metabolism to 20α-P_4 rather than by reduced synthesis of P_4. Another unusual feature of steroid metabolism is the dependence of ovarian oestrogen synthesis on substrate (androstenedione) secreted by the placenta. The luteolytic signal arises from the gravid uterus, but whether it is the result of withdrawal of luteotrophin or release of a luteolysin ($PGF_{2\alpha}$) is uncertain. The resulting fall in P_4 values while ovarian oestrogen secretion is maintained is associated with a sharp rise in oxytocin receptors, α_1-adrenoceptors and relaxin, all of which are necessary for the timely onset and progress of parturition. Delivery of the litter is dependent on release of oxytocin during the expulsive phase of labour. The role of prostanoids in the onset of parturition is uncertain because they have little oxytocic activity *in vivo* and the part they play in luteolysis is not established, but they may contribute to the formation of gap junctions, oxytocin receptors and α_1-adrenoceptors and to the release of relaxin.

9.10 GUINEA PIG

9.10.1 INTRODUCTION

Pregnancy in the guinea pig has many features in common with human pregnancy and the mechanism of initiation of parturition (65 days) is likewise unknown. The paracrine hypothesis that is currently under active investigation in humans remains unexplored in the guinea pig.

9.10.2 ROLE OF THE FETUS

The results of experiments attempting to establish a fetal role in parturition are conflicting and cannot be fitted into a plausible hypothesis. The concentration of cortisol in fetal plasma increases markedly in the last week of pregnancy due to both rising levels of cortisol-binding globulin and increased rate of secretion (Dalle and Delost, 1980). The fraction of free cortisol is proportionately greater, suggesting that it could serve as a stimulus to parturition. However, a daily injection of a large dose of dexamethasone into each fetus in three animals during the last week of pregnancy did not advance the time of parturition (Illingworth *et al.*, 1974). Furthermore, destruction of the fetal pituitary or pituitary stalk has no effect on pregnancy length (Donovan and Peddie, 1973), although fetal adrenal growth is impaired. The placenta is freely permeable to cortisol and the rise in fetal levels of cortisol parallels that in the maternal plasma (Dalle and Delost, 1976). The administration of dexamethasone to the mother has no effect on the time of parturition, although passage into the fetus is likely (Illingworth *et al.*, 1974). Paradoxically, preliminary experiments in which the fetal midline basal hypothalamus was lesioned were followed by abortion (Donovan and Peddie, 1973). Available evidence makes it clear that the prepartum increase in fetal cortisol values does not trigger parturition, but the role of the fetal hypothalamus and pituitary is ambiguous.

9.10.3 PROGESTERONE

Pregnancy becomes independent of the CL in mid-pregnancy when placental production of

P_4 is sufficient to maintain pregnancy. Ovariectomy before 30 days causes abortion but delivery at term occurs when ovariectomy is performed after 30 days (Csapo *et al.*, 1981). However, the CL remains functional throughout pregnancy and circulating concentrations of P_4 fall by 40% after ovariectomy at term (Batra and Thorbert, 1981). High P_4 concentrations, with much P_4 bound to a unique binding globulin, are maintained to term and do not fall before the onset of labour (Heap and Illingworth, 1974).

Doubt has been expressed that progesterone is a myometrial regulatory agent in the guinea pig. Myometrial activity continues throughout pregnancy despite high levels of P_4. Exogenous P_4, even when placed in the uterus, is without effect on the duration of pregnancy or on the mechanical activity of the uterus (Porter, 1970, 1971). However, the refractoriness of the uterus could be attributable to full occupancy of P_4 receptors. P_4 inhibits and oestrogen stimulates receptor formation (Sumida *et al.*, 1988); accordingly, the very high P_4–oestrogen ratio prevailing throughout pregnancy is likely to be associated with a low concentration and a high occupancy of receptors. Experiments with P_4 antagonists leave little doubt that the maintenance of pregnancy is dependent on placental P_4 because such treatment causes abortion after 40 days' gestation (Elger *et al.*, 1986). This does not necessarily imply a direct action of P_4 on the myometrium as the effect of P_4 antagonists could be mediated, for example, by the release of PGs.

9.10.4 OESTROGENS

Concentrations of oestrogen in the maternal circulation are very low in the second half of pregnancy and fall during the week before term (Illingworth *et al.*, 1974). The levels are unchanged by bilateral ovariectomy (Batra and Thorbert, 1981), suggesting that the site of oestrogen synthesis is within the uterus. The placenta has little aromatase activity (Ainsworth and Ryan, 1966) and the site of synthesis appears to be intrafetal because Challis and Illingworth (1972) demonstrated that the concentration of labelled oestrogen after injection of radioactive androstenedione was higher in the umbilical artery than in the umbilical vein. Much of the radioactivity was associated with the 16α-hydroxylated products, oestriol and epioestriol.

Administration of oestrogen does not induce labour; indeed, implants of oestradiol or stilboestrol delay the onset of labour and cause a high incidence of fetal death (Illingworth *et al.*, 1974). The mechanism of delayed delivery is uncertain but could be attributable to induction of P_4 receptors (Sumida *et al.*, 1988) by oestrogen. The concentration of nuclear oestrogen receptors expressed per mg of DNA in the myometrium increases from 50 days to term but remains unchanged when expressed per mg of protein (Alexandrova and Soloff, 1980d).

9.10.5 OXYTOCIN

Oxytocin is undetectable in the maternal circulation before the expulsive phase of labour, when it is released in substantial amounts (Burton *et al.*, 1974). The plasma of the firstborn fetus contains no oxytocin, but subsequent fetuses have concentrations approaching those of the mother. Since the placenta is permeable to oxytocin, this suggests a maternal origin for the oxytocin in the fetal circulation. Oxytocin receptor concentrations in the myometrium rise throughout pregnancy to 60 days and plateau thereafter until term (Alexandrova and Soloff, 1980d). This pattern is mirrored by sensitivity to oxytocin, which increases 50-fold from early to late pregnancy (Bell, 1941). The absence of detectable oxytocin in plasma or an increase in myometrial oxytocin receptors before parturition is inconsistent with a role for oxytocin in the initiation of parturition, but until decidua has been studied the

possibility of a prepartum increase in receptors in that tissue cannot be ruled out.

9.10.6 RELAXIN

Relaxin immunoreactivity in guinea pig plasma (measured by RIA using an antibody against pig relaxin) during late pregnancy does not show any consistent pattern between animals. Relaxin concentrations rise in mid- to late pregnancy in some animals, and in others values rise shortly before parturition (Boyd *et al.*, 1981). Lengthening of the pubic ligaments is preceded by increased plasma concentrations of relaxin in most, but not all, animals.

Circulating relaxin may arise from the endometrial glands, where increasing quantities can be demonstrated by immunocytochemistry as pregnancy progresses (Pardo *et al.*, 1980). Treatment of ovariectomized guinea pigs with oestrogen and P_4 not only induces accumulation of relaxin in endometrial glands but also reproduces the marked relaxation of the pelvic ligaments that occurs in response to treatment with relaxin (Pardo and Larkin 1982). Injections of pig relaxin inhibit spontaneous uterine activity in non-pregnant guinea pigs and, because P_4 apparently lacks inhibitory effects, Porter (1972) suggested that relaxin is the hormone mainly responsible for uterine quiescence in this species.

9.10.7 CATECHOLAMINES

The total content of myometrial α_1-adrenoceptors increases 34-fold in pregnancy, but this is attributable to growth of the uterus and not to an increase in concentration (Arkinstall and Jones, 1989). Together with the observation that the excitatory response to noradrenaline declines towards term (Carter, 1985), this suggests that α_1-adrenergic stimulation is unlikely to contribute to the initiation of parturition in the guinea pig.

9.10.8 EICOSANOIDS

Information about the synthesis of prostanoids in the pregnant guinea pig is almost completely lacking. Plasma concentrations of PGF in peripheral plasma remain unchanged with the onset of parturition, but there is a twofold increase in PGF and 6-keto-$PGF_{1\alpha}$ in vena caval plasma (Kendall, 1977). PGE_2 and its metabolites have not been measured. The endometrium produces significant quantities of PGs at least in early pregnancy (Poyser, 1984) which may be important in activating the myometrium; strips of circular muscle from pregnant animals become quiescent *in vitro* unless the endometrium is left intact (H.A. Coleman and Parkington, 1988). Daily subcutaneous injections of $PGF_{2\alpha}$ or PGE_2 at 43 days induce abortion, but a high proportion of fetuses are dead when expelled (Elger, 1979); PGs therefore differ from oxytocin, which induces the abortion of living fetuses.

9.10.9 SUMMARY

It is difficult to construct a satisfactory hypothesis for the mechanism that initiates parturition in guinea pigs. Clearly, parturition is not dependent on luteolysis, and available evidence does not support an active role for oxytocin. Insufficient information is available to assess the possible role of prostanoids.

9.11 DOG

9.11.1 INTRODUCTION

Reported studies of pregnancy and parturition are almost exclusively related to laboratory-bred beagles. P_4 and oestrogen levels in Labrador bitches are similar to those of beagles, and there is no reason to expect major differences in the mechanism of parturition within the canine species. The dog belongs to the large group of mammals in which parturition is preceded by luteolysis.

9.11.2 ROLE OF THE FETUS

The contribution of the fetus to the onset of parturition has not been studied. Pregnancy and pseudopregnancy are of the same duration (65–66 days) but, although differences in the endocrine milieu of the two are rather subtle, it is clear that the presence of the conceptus is expressed in CL function. Fetal corticosteroids are unlikely to play a part because administration of dexamethasone from 45 days leads to fetal death but not parturition (Austad *et al.*, 1976).

9.11.3 PROGESTERONE

Maintenance of pregnancy is dependent on P_4, and ovariectomy at 50–55 days is followed by parturition (Sokolowski, 1971). In both the pregnant and pseudopregnant animals, P_4 concentrations rise to high values (25 ng/ml) by 25 days and then fall progressively to about 3 ng/ml 3 days before parturition (Concannon *et al.*, 1975). The concentration continues to fall slowly in the pseudopregnant bitch, whereas it falls abruptly in the pregnant animal to about 1 ng/ml on the day of parturition (Figure 9.18).

P_4 implants at 60 days prevents parturition at term and results in prolonged pregnancy and death of the mother (Concannon *et al.*, 1977). Pregnancy is maintained when plasma P_4 concentrations exceed 2 ng/ml. Luteolysis terminates luteal function abruptly about 48 h before parturition. The rapid withdrawal of progestational activity immediately preceding parturition is apparent in hypothermia and a sharp rise in plasma prolactin, both of which begin shortly after the fall in P_4 levels (Concannon *et al.*, 1978).

9.11.4 OESTROGEN

Oestrogen concentrations remain steady through the second half of pregnancy at

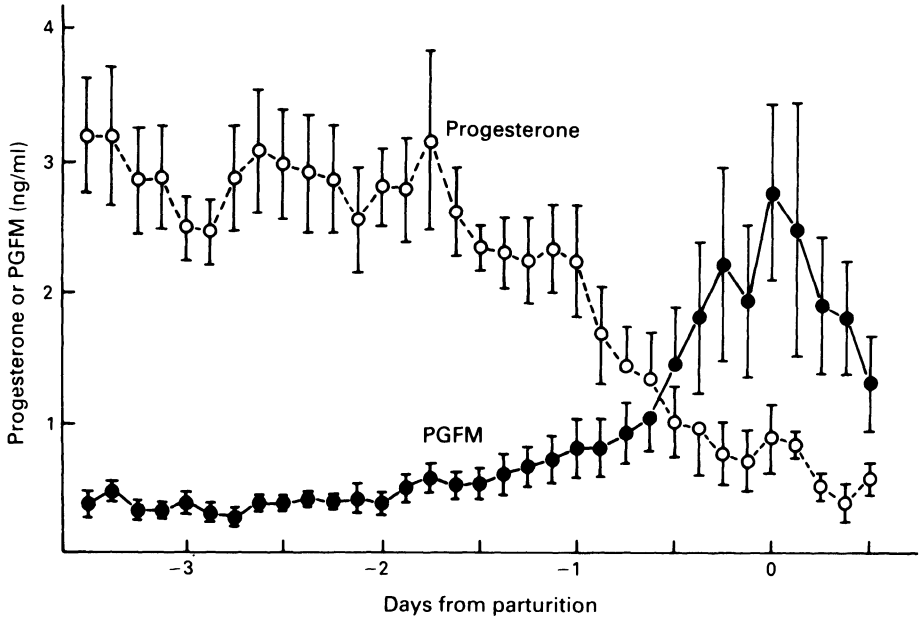

Figure 9.18 Concentrations of progesterone and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) in peripheral plasma of bitches around parturition. (Reproduced with permission from Concannon *et al.*, 1988.)

about twice the values of the non-pregnant cycle (Concannon *et al.*, 1975) then fall sharply during the 2 days prepartum to reach non-pregnant values by the day of parturition. Immunoreactive plasma oestrogen is mainly oestradiol-17β (Austad *et al.*, 1976). The source of oestrogen is unknown, but the prepartum fall in concentrations which coincides with a similar fall in P_4 points strongly to the ovary.

9.11.5 RELAXIN

Plasma relaxin concentrations rise in the second half of pregnancy in intact and ovariectomized animals in which pregnancy is maintained with injections of 17α-ethyl-19-nortestosterone, although values are lower in the latter group (Steinetz *et al.*, 1989). These findings suggest that relaxin is secreted by the ovaries and the uterus. Parturition is not heralded by any marked changes in relaxin values.

9.11.6 PROSTAGLANDINS

The concentration of PGFM in peripheral plasma increases fivefold during the 3 days before parturition (Concannon *et al.*, 1988). The close temporal relationship of rising PGFM and falling P_4 values is consistent with a luteolytic role for $PGF_{2\alpha}$. Administration of $PGF_{2\alpha}$ induces luteolysis (Concannon and Hansel, 1977), but whether PG antagonists prevent luteolysis is unknown.

9.11.7 SUMMARY

The initiation of parturition in the dog is almost certainly a consequence of luteolysis, and the luteolytic agent is probably $PGF_{2\alpha}$, but the stimulus to release of $PGF_{2\alpha}$ is unknown. The hormonal changes at parturition are unusual in that both P_4 and oestrogen fall sharply at luteolysis and there is little or no change in the oestrogen–P_4 ratio. The trigger to parturition therefore appears to be exclusively a fall in P_4 concentrations.

9.12 RABBIT

The maintenance of pregnancy to term (31 days) in rabbits has been known to depend on the presence of functional CL secreting P_4 since the classical experiments of Fraenkel (1910) and Corner (1928). Luteolysis precedes parturition and is its cause, but the factors leading to luteolysis, which must be considered to be the primary step in the mechanism initiating parturition, remain uncertain.

9.12.1 ROLE OF THE FETUS

Bauman and Dziuk (1975) decapitated all fetuses in the litter at 18–21 days: pregnancy length was shortened by 2 days in the does carrying decapitated fetuses, but only about 10% of the fetuses were subsequently recovered and no sham operations were included, making interpretation of the results difficult. Chiboka *et al.* (1977) removed the fetuses at 25 days and observed normal delivery of placentas at term preceded by normal nest-building behaviour. When fetectomy was performed at 21 days, placentas were retained until 36 days. The results were interpreted as indicating that the fetuses programme the placenta before 25 days of gestation but thereafter the placenta is autonomous in determining the time of parturition.

9.12.2 PROGESTERONE

The contribution of the placenta to the raised concentration of P_4 in the maternal circulation was investigated by Thau and Lanman (1974). Pregnant does were ovariectomized at 21 days and pregnancy was maintained by injections of MPA, which does not cross-react in the RIA of P_4. The concentration of P_4 fell promptly to non-pregnant values, indicating that the CL is the only significant source of the raised levels of P_4 in pregnancy.

The concentration of P_4 in maternal peripheral plasma follows a well-defined pattern for which there is general agreement (Kendall and Liggins, 1972; Challis *et al.*, 1973b; Singh and Adams, 1978; Lau *et al.*, 1982): values rise rapidly during the first 8 days of pregnancy, reach peak values at 14 days, fall slightly to a plateau until 29 days then decline rapidly to reach low levels a few hours before parturition. The concentration of 20α-P_4 is approximately the same as that of P_4 and follows the same pattern through pregnancy (Fuchs, 1978). The concentration of P_4 in the myometrium closely parallels that of the plasma throughout pregnancy and the ratio of myometrial–plasma concentration approximates unity throughout most of pregnancy but falls slightly below unity near term. The concentration of P_4 receptors in myometrium and endometrium has been studied by Quirk and Currie (1984), who found that the nuclear P_4 receptor concentration in myometrium is inversely related to the plasma concentration of P_4, and attributed this surprising result to stimulation of P_4 receptor formation by the rising ratio of oestrogen–P_4 as plasma concentrations of P_4 (but not of oestrogen) fall prepartum. The concentration of receptors in endometrium does not rise as P_4 levels fall. Quirk and Currie (1984) concluded that the level of P_4 receptors in rabbit myometrium does not reflect the physiological response to P_4. Evidence that the secretion of P_4 by the CL is dependent on luteotrophin(s) secreted by the placenta has been provided by experiments in which fetectomy was performed at 15 days and the placentas were left in place (Klein, 1967). At necropsy at 20 days, the CL had not regressed. Transplantation of placentas to the body wall or abdominal cavity prolongs the lifespan of the CL nearly to term (Chu *et al.*, 1946). The nature of the placental luteotrophin and the extent to which maternal pituitary LH is part of the luteotrophic complex in the rabbit are uncertain, as also is whether luteolysis normally results from withdrawal of luteotrophic support, from the action of a luteolysin or from both effects. Dislocation of the placentas is followed by luteolysis and abortion (Csapo, 1969). Although this experiment is open to several interpretations, including release of prostanoids resulting from trauma, loss of placental luteotrophin is probably the most likely explanation since leaving a single placenta intact maintains pregnancy yet is unlikely to reduce significantly the extent of trauma.

Inhibition of P_4 synthesis by oral administration of isoxazol, a 3β-HSD inhibitor, at 25 days is followed by increasing sensitivity to oxytocin and oxytocin-induced abortion in most animals within 2 days (Bernard *et al.*, 1980). The claim that P_4 concentrations in the uterine vein in these experiments fell as a result of treatment is doubtful since the post-treatment blood sample was collected at post-partum autopsy. The study needs to be repeated with other inhibitors and improved sampling procedures.

9.12.3 OESTROGEN

The placenta probably does not secrete oestrogen (Ainsworth and Ryan, 1966) and the ovarian follicles are the sole source. Loss of the follicles either by their removal, by inducing ovulation or by hypophysectomy causes abortion which can be prevented by the administration of oestrogen (Gadsby *et al.*, 1983). Withdrawal of oestrogen is followed rapidly by a fall in circulating progesterone to low levels which can be fully reversed by oestrogen treatment 24 h after oestrogen withdrawal provided that abortion is prevented by administering medroxyprogesterone (Gadsby *et al.*, 1983). Thus, it appears that the putative placental luteotrophin depends on follicular oestrogen for either its action or its production.

A fall in oestrogen production is a potential physiological mechanism for initiating luteolysis and parturition, but studies of plasma oestrogen concentrations are not supportive.

Both oestrogen and oestradiol-17β levels remain within a narrow range throughout pregnancy and show no significant trend at term (Challis *et al.* 1973b; Lau *et al.*, 1982).

9.12.4 OXYTOCIN

The sensitivity to oxytocin increases dramatically prepartum (Fuchs, 1964; Porter and Schofield, 1966), and a small single intravenous dose of oxytocin leads to rapid expulsion of the litter after 30 days, although the uterus is refractory to oxytocin until 25 days. Myometrial contractions occur during delivery in the non-pregnant horn of unilaterally pregnant animals (Fuchs, 1964), suggesting a circulating stimulus, presumed to be oxytocin. Further support for a systemic stimulus was reported by Wagner (1974) who observed electrical activity synchronous with parturitional uterine activity in myometrial transplants in ear chambers. Electrical stimulation of the infundibulum and the median eminence causes parturition provided that the pregnancy is within 24 h of term (Lincoln, 1971). Milk ejection accompanying the electrical stimulation lends strong support to oxytocin release as the stimulus to parturition. The extracellular electrical activity of magnocellular neurones was recorded in unanaesthetized rabbits during parturition by O'Byrne *et al.* (1986). Oxytocin neurones were differentiated from vasopressin neurones by their response to suckling. During parturition, 5–14 discrete bursts of firing were seen, representing a three- to 100-fold increase in the rate of firing compared with prepartum. The increased electrical activity was accompanied by a marked increase in circulating oxytocin, leaving no doubt that the expulsive phase of labour is the consequence of oxytocin release. An obligatory role of oxytocin release to complete parturition is suggested by the prolongation of pregnancy that is associated with high cervical cord transection (Beyer and Mena, 1970).

The mechanism underlying the rapid increase in the sensitivity of the prepartum uterus to oxytocin was elucidated by Reimer *et al.* (1986). They measured the oxytocin receptor concentrations in myometrium each day near term after determining the sensitivity to injections of oxytocin. In addition, they measured contractile responses to oxytocin *in vitro* at 30 days. The response to oxytocin increased at least fourfold between 30 and 31 days of pregnancy, and this increased sensitivity was accompanied by a 10-fold increase in oxytocin binding sites in both decidua and myometrium. The stimulus to the formation of oxytocin receptors in rabbits has not been investigated but may be similar to that in the rat, i.e. inhibited by P_4 and stimulated by oestrogen (Fuchs *et al.*, 1983b). Whether the effects of ovarian hormones are direct or are mediated by other agents such as prostaglandins is uncertain. In the ovariectomized, non-pregnant rabbit, oxytocin stimulates the release of $PGF_{2\alpha}$ from endometrium but not from myometrium (Small *et al.*, 1978). Treatment with oestrogen increases oxytocin-stimulated release of $PGF_{2\alpha}$ and P_4 completely inhibits the response to oestrogen.

9.12.5 CORTICOSTEROIDS

The concentration of cortisol in fetal plasma rises progressively from 24 days to term, raising the possibility that fetal cortisol is an important signal to the initiation of parturition (Mulay *et al.*, 1973; Baldwin and Stabenfeldt, 1974). When cortisol (Nathanielsz and Abel, 1972, 1973; Abel *et al.*, 1973) or dexamethasone (Adams and Wagner, 1969; Kendall and Liggins, 1972; Challis *et al.*, 1975) was given to does at 21–25 days of pregnancy, delivery of pups after 72 h accompanied by nest-building activity and lactation was noted consistently. Induced parturition is preceded by a fall in P_4 concentrations of a similar magnitude to that at normal term, but a rise in plasma concentration of $PGF_{2\alpha}$, which nor-

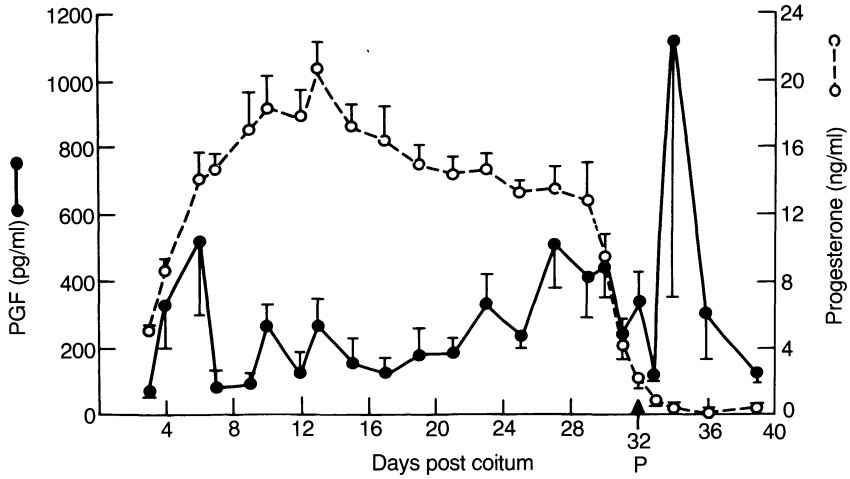

Figure 9.19 Concentration of progesterone and PGF in the peripheral plasma of pregnant rabbits. (Reproduced with permission from Challis *et al.*, 1973a.)

mally accompanies or precedes the fall in P_4, is not present and the response to dexamethasone is not inhibited by concurrent treatment with indomethacin (Challis *et al.*, 1975). Challis *et al.* (1975) proposed that the findings are consistent with a direct action of corticosteroid on the ovary, which would rule out a physiological role for fetal cortisol since it does not have access to the maternal ovary. An alternative explanation is that corticosteroid suppresses the putative placental luteotrophin, which could be influenced by fetal cortisol, but administered corticosteroids might have a physiological or pharmacological action on the placenta. Further experiments, including stimulation of the fetal adrenals with ACTH, are required before a role in parturition can be confidently attributed to rising concentrations of fetal cortisol before term.

9.12.6 RELAXIN

Relaxin has been purified from the placenta (P.A. Fields *et al.*, 1982) but the source of circulating relaxin is unknown.

9.12.7 EICOSANOIDS

Information about the maternal plasma concentrations of prostanoids preceding parturition is limited. Assays of PGFM or a stable metabolite of PGE_2 in serial samples near term have not been reported. Challis *et al.* (1973a) found that the peripheral concentration of PGF increased significantly from 21 to 30 days in samples taken on alternate days (Figure 9.19).

In three animals sampled daily from 29 days values were raised on days 27–30 in two and not in the third, in which P_4 levels did not fall until 30 days. These results were not confirmed by Lytton and Poyser (1982), who found a peak of $PGF_{2\alpha}$ in uterine vein blood at 25 days, but concentrations remained low in arterial and peripheral venous blood to term. A physiological role of $PGF_{2\alpha}$ in luteolysis is not supported by experiments in which pregnant rabbits were given indomethacin in late pregnancy (Challis *et al.*, 1975). The treatment prolonged pregnancy by 1 day but did not prevent luteolysis and P_4 concentration fell over the same time course as it did in controls. While there is doubt

whether release of $PGF_{2\alpha}$ precedes luteolysis, and that endogenous $PGF_{2\alpha}$ is luteolytic, infusion of $PGF_{2\alpha}$ causes an irreversible fall in P_4 within 2 h (Challis *et al.*, 1974c; Laudanski *et al.*, 1979). Continuous intra-aortic infusion of $PGF_{2\alpha}$ for a minimum period of 8 h at 21 days is followed by delivery after a mean latent period of 38 h (Nathanielsz *et al.*, 1973). Inhibition of P_4 synthesis by administering trilostane, a 3β-HSD inhibitor, as a single intravenous bolus at 28 days causes an immediate fall in P_4 and delivery after 30 h but the concentration of PGFM in peripheral blood remains low until labour starts, when it rises sharply (Makimura *et al.*, 1984).

M.H. Block *et al.* (1985) showed that placental microsomes in late pregnancy efficiently metabolize labelled arachidonic acid to PGE_2 and $PGF_{2\alpha}$, PGD_2 and TXA_2, but little PGI_2, and the cytosolic fraction generates a variety of lipoxygenase products.

9.12.8 SUMMARY

Parturition is immediately preceded by luteolysis and a marked fall in P_4 concentrations, but the cause of luteolysis is not known. Although administered $PGF_{2\alpha}$ causes luteolysis, indomethacin fails to block luteolysis, suggesting that luteolysis is not normally initiated by release of $PGF_{2\alpha}$. Parturition is readily provoked by oxytocin at term but the uterus is insensitive to oxytocin earlier in gestation. The increased sensitivity to oxytocin is probably due to a marked increase in oxytocin receptors that accompanies luteolysis and is likely to result from the fall in P_4. Luteolysis is probably due to withdrawal of luteotrophic support rather than the release of a luteolysin, but the nature of the luteotrophin(s) and the stimulus to its withdrawal are unknown.

9.13 CONCLUSIONS

The past 25 years have seen enormous strides in the understanding of the mechanism of initiation of parturition. In some species, sheep in particular, knowledge has reached a level of sophistication similar to that in many other physiological systems. In others, primates in particular, understanding of how the system does **not** work is substantial, but solid hypotheses capable of being tested are lacking. Clearly, wide diversity is a characteristic of mammalian parturition and it is difficult to discern an evolutionary pathway. To some extent, the needs of the newborn can be linked teleologically to the type of mechanism in a given species; precocial species having a mature newborn capable of fending for itself immediately after birth are well served by a system where the fetus has firm control of the time of birth, whereas the more immature newborn dependent for survival on prolonged mothering tolerates systems that are less tightly tied to the fetus and maternal influences are more readily seen. Even in sheep, however, maternal circadian rhythms probably contribute to the hour of birth if not the day. In evolutionary terms, whether the degree of maturity at birth is a cause or a consequence of a particular system of determining the time of birth is moot.

The widely differing systems across the species tend to converge towards the end of their respective pathways so that the roles played by agents such as prostaglandins and oxytocin that activate the uterus have much in common. The extent to which the pathways share a common origin is less clear. In general, opinions are divided on whether the signal is complex and multifactorial, deriving from input from many maturing organs or comes from one or more specific genes that function as a clock. Such a clock need not be absolutely accurate since the signal may not only initiate parturition but also accelerate maturation of those organs on which postnatal survival is most dependent.

For the moment, it seems that classic endocrine and physiological techniques have nearly reached their limit and that further progress is likely to come from investigations

at a cellular level. Hopefully, however, the wheel will turn full circle and it will be possible in due course to return to the whole animal armed with testable hypotheses derived from the study of cells.

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REFERENCES

Abel, M., Taurog, J. and Nathanielsz, P.W. (1973) A comparison of the luteolytic effect of $PGF_{2\alpha}$ and cortisol in the pregnant rabbit. *Prostaglandins*, **4**, 433–440.

Abel, M.H., Smith, S.K. and Baird, D.T. (1980) Suppression of concentration of endometrial prostaglandin in early intra-uterine and ectopic pregnancy in women. *Journal of Endocrinology*, **85**, 379–386.

Adams, W.M. and Wagner, W.C. (1969) The elective induction of parturition in cattle, sheep and rabbits. *Journal of the American Veterinary Medical Association*, **154**, 1396–1397.

Adams, W.M. and Wagner, W.C. (1970) The role of corticoids in parturition. *Biology of Reproduction*, **3**, 223–228.

Ainsworth, L. (1972) The cleavage of steroid sulphates by sheep and pig foetal liver, foetal kidney and placental preparation *in vitro*. *Steroids*, **19**, 741–750.

Ainsworth, L. and Ryan, K.J. (1966) Steroid hormone transformations by endocrine organs from pregnant mammals. I. Oestrogen biosynthesis by mammalian placental preparations *in vitro*. *Endocrinology*, **79**, 875–883.

Ainsworth, L. and Ryan, K.J.U. (1967) Steroid hormone transformations by endocrine organs from pregnant mammals. II. Formation and metabolism of progesterone by bovine and sheep placental preparations *in vitro*. *Endocrinology*, **81**, 1349–1355.

Ainsworth, L. and Ryan, K.J. (1969) Steroid hormone transformations by endocrine organs from pregnant mammals. III. Biosynthesis and metabolism of progesterone by the mare placenta *in vitro*. *Endocrinology*, **84**, 91–97.

Ainsworth, L. and Ryan, K.J. (1970) Steroid hormone transformations by endocrine organs from pregnant mammals. VI. The conversion of 4-androstene-3,17-dione to estrogens by goat placental preparations *in vitro*. *Steroids*, **14**, 553.

Ainsworth, L., Daenen, M. and Ryan, K.J. (1969) Steroid transformations by endocrine organs from pregnant mammals. IV. Biosynthesis and metabolism of estrogens and progesterone by primate placental preparation *in vitro*. *Endocrinology*, **84**, 1421–1429.

Aitken, J.W. (1971) Aspirin and indomethacin prolong parturition in rats: evidence that prostaglandins contribute to expulsion of foetus. *Nature*, **240**, 21–25.

Akerlund, M., Stromberg, P., Hanksson, A., Andersen, L.F., Lyndrup, J., Trojnar, J. and Melin, P. (1987) Inhibition of uterine contractions of premature labour with an oxytocin analogue. Results from a pilot study. *British Journal of Obstetrics and Gynaecology*, **94**, 1040–1044.

Akinlosotu, B.A., Diehl, J.R. and Gimenez, T. (1986) Sparing effects of intrauterine treatment with PGE_2 on luteal function in cycling gilts. *Prostaglandins*, **32**, 291–297.

Albrecht, E.D. (1980) A role for estrogen in progesterone production during baboon pregnancy. *American Journal of Obstetrics and Gynecology*, **136**, 569–574.

Albrecht, E.D., Haskins, A.L. and Pepe, G.J. (1980) The influence of fetectomy at midgestation upon the serum concentrations of progesterone, estrone, and estradiol in baboons. *Endocrinology*, **107**, 766–770.

Alexandrova, M. and Soloff, M.S. (1980a) Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term. *Endocrinology*, **106**, 730–735.

Alexandrova, M. and Soloff, M.S. (1980b) Oxytocin receptors and parturition. II. Concentrations of receptors for oxytocin and estrogen in the gravid and nongravid uterus at term. *Endocrinology*, **106**, 736–738.

Alexandrova, M. and Soloff, M.S. (1980c) Oxytocin receptors and parturition. III. Increases in estrogen receptor and oxytocin receptor concentrations in the rat myometrium during prostaglandin $F_{2\alpha}$-induced abortion. *Endocrinology*, **106**, 739–743.

Alexandrova, M. and Soloff, M.S. (1980d)

Oxytocin receptors and parturition in the guinea pig. *Biology of Reproduction*, **22**, 1106–1111.

Allen, W.R., Chard, T. and Forsling, M.L. (1973) Peripheral plasma levels of oxytocin and vasopressin in the mare during parturition. *Journal of Endocrinology*, **57**, 175–176.

Alm, C.C., Sullivan, J.J. and First, N.L. (1975) Induction of premature parturition by parenteral administration of dexamethasone in the mare. *Journal of the American Veterinary Medicine Association*, **165**, 721–722.

Anderson, A.B.M., Pierrepoint, C.G., Jones, T., Griffiths, K. and Turnbull, A.C. (1970) Steroid biosynthesis *in vitro* by foetal and adult sheep adrenal tissue. *Journal of Reproduction and Fertility*, **22**, 99–107.

Anderson, A.B.M., Flint, A.P.F. and Turnbull, A.C. (1975) Mechanism of action of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. *Journal of Endocrinology*, **66**, 61–70.

Anderson, L.L., Dyck, G.W. and Rathmacher, R.P. (1966) Pituitary gonadotrophic activities following luteal enucleation in the pig. *Endocrinology*, **78**, 897–900.

Anderson, L.L., Dyck, G.W., Mori, H., Henricks, D.M. and Melampy, R.M. (1967) Ovarian function in pigs following hypophysial stalk transection or hypophysectomy. *American Journal of Physiology*, **212**, 1118–1194.

Anderson, L.L., Adair, V., Stomer, M.H. and McDonald, W.G. (1983) Relaxin production and release after hysterectomy in the pig. *Endocrinology*, **113**, 677–686.

Anderson, N.G., Curet, L.B. and Colas, A.E. (1978a) Changes in C_{21}-steroid metabolism by ovine placentas during cortisol administration. *Biology of Reproduction*, **18**, 652–657.

Anderson, N.G., Curet, L.B. and Colas, A.E. (1978b) Changes in C_{19}-steroid metabolism by ovine placentas during cortisol administration. *Biology of Reproduction*, **18**, 643–651.

Andrianakis, P., Walker, D.W., Ralph, M.M. and Thorburn, G.D. (1989a) Effects of hyperthermia on fetal and maternal plasma prostaglandin concentrations and uterine activity in sheep. *Prostaglandins*, **38**, 541–555.

Andrianakis, P., Walker, D.W., Ralph, M.M. and Thorburn, G.D. (1989b) Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions. *American Journal of Obstetrics and Gynecology*, **161**, 241–247.

Angle, M., Maki, N. and Johnston, J.M. (1988) Bioactive metabolites of glycerophospholipid metabolism in relation to parturition, in *The Onset of Labor: Cellular and integrative mechanisms*. (eds D. McNellis, J. Challis, P. MacDonald, P.W. Nathanielsz and J. Roberts), Perinatology Press, Ithaca, pp. 125–138.

Arkinstall, S.J. and Jones, C.T. (1989) Myometrial alpha 1-adrenoceptors in pregnant guinea pigs: their distribution and increased number. *American Journal of Physiology*, **256**, E215–E220.

Ash, R.W. and Heap, R.B. (1975) Oestrogen, progesterone and corticosteroid concentration in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. *Journal of Endocrinology*, **64**, 141–154.

Austad, R., Lunde, A. and Sjaastad, O.V. (1976) Peripheral plasma levels of oestradiol-17β and progesterone in the bitch during the oestrus cycle, in normal pregnancy and after dexamethasone treatment. *Journal of Reproduction and Fertility*, **46**, 129–136.

Baldwin, D.M. and Stabenfeldt, G.H. (1974) Plasma levels of progesterone, cortisol, and corticosterone in the pregnant rabbit. *Biology of Reproduction*, **10**, 495–501.

Baldwin, D.M. and Stabenfeldt, G.H. (1975) Endocrine changes in the pig during late pregnancy, parturition and lactation. *Biology of Reproduction*, **12**, 508–515.

Balfour, W.E., Comline, R.S. and Short, R.V. (1957) Secretion of progesterone by the adrenal gland. *Nature (London)*, **180**, 1480–1481.

Ball, G.D. and Day, B.N. (1982a) Bilateral luteal maintenance in unilaterally pregnant pigs with infusions of embryonic extracts. *Journal of Animal Science*, **54**, 142–149.

Ball, G.D. and Day, B.N. (1982b) Local effects of $PGF_{2\alpha}$ and embryonic extracts on luteal function in swine. *Journal of Animal Science*, **54**, 150–154.

Ballard, P.L. and Liggins, G.C. (1982) Glucocorticoid activity in cord serum after prenatal corticosteroid therapy: Comparison of hydrocortisone and betamethasone regimens. *Journal of Pediatrics*, **101**, 468–470.

Ballard, P.L., Kitterman, J.A., Bland, R.D., Clyman, R.I., Gluckman, P.D., Platzker, A.C., Kaplan, S.L. and Grumbach, M.M. (1982) Ontogeny and regulation of corticosteroid binding globulin capacity in plasma of fetal and newborn lambs. *Endocrinology*, **110**, 359–366.

Barcikowski, B., Carlson, J., Wilson, L. and McCracken, J.A. (1974) The effect of endogen-

ous and exogenous oestradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. *Endocrinology*, **95**, 1340–1349.

Barnafi, L. and Larraguibel, R. (1974) The *in vitro* effect of progesterone and oestrogens on the spontaneous and oxytocin-induced activity of the human myometrium. *Acta Endocrinologica*, **76**, 172–177.

Barnes, R.J., Comline, R.S. and Silver, M. (1974) Foetal and maternal progesterone concentrations in the sow. *Journal of Endocrinology*, **62**, 419–420.

Barnes, R.J., Comline, R.S., Jeffcott, L.B., Mitchell, M.D., Rossdale, P.D. and Silver, M. (1978) Foetal and maternal concentrations of 13,14-dihydro-15-oxo-prostaglandin F in the mare during late pregnancy and at parturition. *Journal of Endocrinology*, **78**, 201–215.

Bartholomeusz, R.K., Bruce, N.W., Martin, C.E. and Hartmann, P.E. (1976) Serial measurements of arterial plasma progesterone levels throughout gestation and parturition in individual rats. *Acta Endocrinologica*, **82**, 436–443.

Bassett, J.M. and Thorburn, G.D. (1969) Foetal plasma corticosteroids and the initiation of parturition in the sheep. *Journal of Endocrinology*, **44**, 285–286.

Bassett, J.M., Oxborrow, T.J., Smith, I.D. and Thorburn, G.D. (1969) The concentration of progesterone in the peripheral plasma of the pregnant ewe. *Journal of Endocrinology*, **45**, 449–457.

Basuray, R., Jaffe, R.C. and Gibori, G. (1983) Role of decidual luteotropin and prolactin in the control of luteal cell receptors for estradiol. *Biology of Reproduction*, **28**, 551–556.

Batra, S. and Bengtsson, L.P. (1978) 17β-estradiol and progesterone concentrations in myometrium of pregnancy and their relationships to concentrations in peripheral plasma. *Journal of Clinical Endocrinology and Metabolism*, **46**, 622–626.

Batra, S.W. and Thorbert, G. (1981) Local placental influence on the uterine concentration of oestradiol-17β and progesterone in the pregnant guinea-pig. *Acta Endocrinologica*, **98**, 302–307.

Battista, P.J., Bell, A.W. and Currie, W.B. (1989) Different mechanisms control placental lactogen and progesterone secretion by ovine placental tissue (abstract). *Journal of Reproduction and Fertility*, Abstract 98.

Baulieu, E.E., Ulmann, A. and Philibert, D. (1987) Contragestion by antiprogestin RU 486: A review. *Archives of Gynecology and Obstetrics*, **241**, 73–85.

Bauman, D.A. and Djuik, P.J. (1975) Rabbit gestation length after fetal decapitation (abstract). *Journal of Animal Science*, p. 343.

Bazer, F.W. and Thatcher, W.W. (1977) Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin $F_{2\alpha}$ by the uterine endometrium. *Prostaglandins*, **14**, 397–401.

Bazer, F.W., Vallet, J.L., Roberts, R.M., Sharp, D.C. and Thatcher, W.W. (1986) Role of conceptus secretory products in establishment of pregnancy. *Journal of Reproduction and Fertility*, **76**, 841–850.

Beck, P., Adler, P., Szlachter, N., Goldsmith, L.T., Steinetz, B.G. and Weiss, G. (1982) Synergistic effect of human relaxin and progesterone on human myometrial contractions. *International Journal of Gynecology and Obstetrics*, **20**, 141–144.

Bedford, C.A., Harrison, F.A. and Heap, R.B. (1972a) The metabolic clearance rate and production rate of progesterone and the conversion of progesterone to 20-hydroxypregn-4-en-3-one in the sheep. *Journal of Endocrinology*, **55**, 105–118.

Bedford, C.A., Challis, J.R., Harrison, F.A. and Heap, R.B. (1972b) The role of oestrogens and progesterone in the onset of parturition in various species. *Journal of Reproduction and Fertility*, Suppl. 16, 1–23.

Bedford, C.A., Harrison, F.A. and Heap, R.B. (1974) Splanchnic, uterine, ovarian and adrenal uptake of progesterone in the pregnant and non-pregnant sheep. *Journal of Endocrinology*, **62**, 277–290.

Behrman, H.R., Yoshinaga, K. and Greep, R.O. (1971) Extra-luteal effects of prostaglandins. *Annals of the New York Academy of Sciences*, **180**, 426–432.

Beier, H.M. (1968) Uteroglobin, a hormone sensitive endometrial protein involved in blastocyst development. *Biochimica et Biophysica Acta*, **160**, 289–294.

Bell, G.H. (1941) The behaviour of the pregnant uterus of the pregnant guinea pig. *Journal of Physiology*, **100**, 263–274.

Belt, W.D., Anderson, L.L., Cavazos, L.F. and Melampy, R.M. (1971) Cytoplasmic granules and relaxin levels in porcine corpora lutea. *Endocrinology*, **89**, 1–10.

Bengtsson, L.P. and Schofield, B.M. (1963) Progesterone and the accomplishment of parturition in the sheep. *Journal of Reproduction and Fertility*, **5**, 423–431.

Bennett, P.R., Elder, M.G. and Myatt, L. (1987) The effects of lipoxygenase metabolites of arachidonic acid on human myometrial contractility. *Prostaglandins*, **33**, 837–844.

Bernard, A. and Ruttner, Z. (1985) Effect of progesterone treatment near term on the onset of labour in rats. *Acta Physiologica Hungarica*, **65**, 367–351.

Bernard, A., Enkola, J. and Salau, G. (1980) Induction of preterm labor in the rabbit by antiprogesterone. *Acta Physiologica Academiae Scientarum Hungaricae*, **55**, 365–369.

Beyer, C. and Mena, F. (1970) Parturition and lactogenesis in rabbits with high spinal cord transection. *Endocrinology*, **87**, 195–197.

Bill, C.H. and Keyes, P.L. (1983) 17β-estradiol maintains normal function of corpora lutea throughout pseudopregnancy in hypophysectomized rabbits. *Biology of Reproduction*, **28**, 608–617.

Billah, M.M. and Johnston, J.M. (1984) Identification of phospholipid platelet-activating factor (1-*O*-alkyl-2-acetyl-sn-glycero-3-phospho choline) in human amniotic fluid and urine. *Biochemical and Biophysical Research Communications*, **113**, 51–58.

Billah, M.M., Di Renzo, G.C., Ban, C., Truong, C.T., Hoffman, D.R., Aneschi, M.M., Bleasdale, J.E. and Johnston, J.M. (1985) Platelet-activating factor metabolism in human amnion and the responses of this tissue to extracellular platelet-activating factor. *Prostaglandins*, **30**, 841–850.

Blackwell, G.J., Carnuccio, R., Di Rosa, M., Flower, R.J., Parente, L. and Persico, P. (1980) Macrocortin: A polypeptide causing the antiphospholipase effect of glucocorticoids. *Nature (London)*, **287**, 147–149.

Block, B.S.B., Liggins, G.C. and Creasy, R.K. (1984) Preterm delivery is not predicted by serial plasma estradiol or progesterone concentration measurements. *American Journal of Obstetrics and Gynecology*, **150**, 716–721.

Block, M.H., McLaughlin, L.L., Crowley, J., Needleman, P. and Morrison, A.R. (1985) Arachidonic acid metabolic pathway of the rabbit placenta. *Prostaglandins*, **29**, 203–216.

Bogdanove, E.M. (1966) Preservation of functional corpora lutea in the rat by estrogen treatment. *Endocrinology*, **79**, 1011–1015.

Boroditsky, R.S., Reyes, F.I., Winter, J.S.D. and Faiman, C. (1978) Maternal serum estrogens and progesterone concentrations preceding normal labor. *Obstetrics and Gynecology*, **51**, 686–691.

Bosc, M.J. (1972) The induction and synchronization of lambing with the aid of dexamethasone. *Journal of Reproduction and Fertility*, **28**, 347–357.

Bosc, M.J. (1973) Modification de la dure de gestation de la truie apres administration d'ACTH aux foetus. *Academie des Sciences, Paris, Comptes Rendus Serie D*, **276**, 3183–3186.

Bosc, M.J. (1974) La parturition provoqué chez les mammifères domestiques, in, *Avortement et Parturition Provoqués* (eds M.J. Bosc, R. Palmer, C. Sureau), Masson, Paris, p. 1.

Bosc, M.J., du Mesnil du Buisson, F. and Locatelli, A. (1974) Mise évidence d'un controle foetal de la parturition chez la truie. Interactions avec la fonction luteale. *Compte Rendu Hebdomadaire des Séances de l'Academie des Sciences (Paris)*, **278**, 1507–1510.

Bosc, M.J., Germain, G., Nicolle, A., Mowen, M., Philibert, D. and Baulieu, E.E. (1987) Control of birth in rats by RU486, an antiprogesterone compound. *Journal of Reproduction and Fertility*, **79**, 1–8.

Bosc, M.J., Duchamp, G. and Rodas, E. (1988) Effet de l'inversion du poulinage au cours du nycthémère chez le chevaux domestique. *Annales de Zootechnic*, **37**, 111–116.

Boyd, S., Kendall, J.Z., Mento, N. and Bryant-Greenwood, G.D. (1981) Relaxin immunoreactivity in plasma during the reproductive cycle of the female guinea pig. *Biology of Reproduction*, **24**, 405–414.

Braverman, M. and Gurpide, E. (1986) *In vitro* effects of human prolactin and oxytocin on sulphatase activity in isolated human decidual cells. *Journal of Clinical Endocrinology and Metabolism*, **63**, 725–729.

Brennecke, S.P., Bessell, C.K., Woods, J.G. and Turnbull, A.C. (1983) A proteinaceous inhibitor of prostaglandin synthase in human amniotic fluid. *Journal of Obstetrics and Gynaecology*, **4**, 73–77.

Brennecke, S.P., Humphreys, J., Bryce, R.L., Teasdale, W.P., Turnbull, A.C. and Mitchell, M.D. (1984) Maternal plasma inhibition of prostaglandin synthase during human pregnancy, parturition and the puerperium. *British Journal of Obstetrics and Gynaecology*, **91**, 349–352.

Brennecke, S.P., Castle, B.M., Demers, L.M. and Turnbull, A.C. (1985) Maternal plasma prostaglandin E_2 metabolite levels during human pregnancy and parturition. *British Journal of Obstetrics and Gynaecology*, **92**, 345–349.

Brinsfield, T.H. (1968) Relationship of endocrine state to spontaneous motility of sheep myometrium *in vitro*. *Journal of Animal Science*, **27**, 439–442.

Brummer, H.C. (1971) Interaction of E prostaglandin and syntocinon on the pregnant human myometrium. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **78**, 305–309.

Bryant-Greenwood, G.D. (1991) The human relaxins: Consensus and dissent. *Molecular and Cellular Endocrinology*, **79**, 125–132.

Bryant-Greenwood, G.D. and Greenwood, F.C. (1988) Postulated roles for luteal or decidual relaxins at parturition in the pregnant sow and women, in *The Physiology and Biochemistry of Human Fetal Membranes*, (ed. B.F. Mitchell), Perinatology Press, Ithaca pp. 141–156.

Bryman, I., Hammerstrom, S., Lindblom, B., Norstrom, A., Wikland, M. and Wiqvist, N. (1985) Leukotrienes and myometrial activity of the term pregnant uterus. *Prostaglandins*, **30**, 907–911.

Bulmer, J. and Sutherland, C.A. (1983) Bone marrow origin of endometrial granulocytes in the early human placental bed. *Journal of Reproductive Immunology*, **5**, 383–387.

Burgess, K.M. (1991) Control of uterine prostaglandin release during pregnancy in the ewe. *PhD Thesis*, Monash University, Melbourne, Australia.

Burgess, K.M., Jenkin, G., Ralph, M.M., Harding, R. and Thorburn, G.D. (1989) Differential effect of oxytocin on uterine prostaglandin release in late ovine pregnancy. *Australian Society for Reproductive Biology*, **21**, Abstract 55.

Burgess, K.M., Ralph, M.M., Jenkin, G. and Thorburn, G.D. (1990) Effect of oxytocin and estradiol on uterine prostaglandin release in non-pregnant and early pregnant ewes. *Biology of Reproduction*, **42**, 822–833.

Burgess, K.M., Jenkin, G., Ralph, M.M. and Thorburn, G.D. (1992) The effect of the antiprogestin RU486 on uterine sensitivity to oxytocin in late pregnant ewes. *Journal of Endocrinology*, **134**, 353–360.

Burton, A.M., Illingworth, D.V., Challis, J.R.G. and McNeilly, A.S. (1974) Placental transfer of oxytocin in the guinea-pig and its release during parturition. *Journal of Endocrinology*, **60**, 499–506.

Buttle, H.L. (1978) The maintenance of pregnancy in hypophysectomized goats. *Journal of Reproduction and Fertility*, **52**, 255–260.

Buttle, H.L. (1983) The luteotrophic complex in hysterectomized and pregnant goats. *Journal of Physiology*, **342**, 399–407.

Bygdeman, M. and Swann, M.L. (1985) Progesterone receptor blockage. Effect on uterine contractility and early pregnancy. *Contraception*, **32**, 45–51.

Caldeyro-Barcia, R. and Sereno, J. (1961) The response of the human uterus to oxytocin throughout pregnancy, in, *Oxytocin* (eds R. Caldeyro-Barcia and H. Heller), Pergamon Press, Oxford, pp 177–202.

Caldwell, R.W., Bellows, R.A., Hall, J.A. and Anthony, R.V. (1990) Administration of pig relaxin to beef heifers 4 or 7 days prepartum. *Journal of Reproduction and Fertility*, **90**, 165–174.

Carnathan, G., Metcalf, L., Cochrane, R. and Nutting, E. (1981) Progesterone depression and pregnancy maintenance during midgestation in rats. *Biology of Reproduction*, **24**, Suppl. 1, 141.

Carnegie, J.A. and Robertson, H.A. (1978) Conjugated and unconjugated estrogens in fetal and maternal fluids of the pregnant ewe: a possible role for estrone sulfate during early pregnancy. *Biology of Reproduction*, **19**, 20221211.

Carsten, M.E. (1979) Calcium accumulation by human uterine microsomal preparations: Effects of progesterone and oxytocin. *American Journal of Obstetrics and Gynecology*, **133**, 598–601.

Carter, A.M. (1985) Excitatory response of guinea-pig myometrium to intravenous noradrenaline declines towards term. *Journal of Reproduction and Fertility*, **75**, 507–512.

Casey, M.L. and MacDonald, P.C. (1988) Decidual activation: The role of prostaglandins in labor, in, *The Onset of Labor: Cellular and Integrative Mechanisms*, (eds D. McNellis, J. Challis, P. MacDonald, P. Nathanielsz and J. Roberts), Perinatology Press, Ithaca pp. 141–156.

Casey, M.L., Cutner, S.J. and Mitchell, M.D. (1983a) Origin of prostanoids in human amniotic fluid: The kidney as a source of amniotic fluid prostanoids. *American Journal of Obstetrics and Gynecology*, **147**, 547–551.

Casey, M.L., MacDonald, P.C. and Mitchell, M.D. (1983b) Stimulation of prostaglandin E_2 production in amnion cells in culture by a substance(s) in human fetal and adult urine.

Biochemical and Biophysical Research Communications, **114**, 1056–1063.

Casey, M.L., MacDonald, P.C. and Mitchell, M.D. (1985) Despite a massive increase in cortisol secretion in women during parturition, there is an equally massive increase in prostaglandin synthesis: a paradox? *Journal of Clinical Investigation*, **75**, 1852–1857.

Casida, L.E. and Warwick, E.J. 1945 The necessity of the corpus luteum for maintenance of pregnancy in the ewe. *Journal of Animal Science*, **4**, 34–36.

Castracane, V.D., Lessing, J., Brenner, S. and Weiss, G. (1985) Relaxin in the pregnant baboon: Evidence for local production in reproductive tissues. *Journal of Clinical Endocrinology and Metabolism*, **60**, 133–136.

Catala, S. and Deis, R.P. (1973) Effect of oestrogen upon parturition, maternal behaviour and lactation in ovariectomized pregnant rats. *Journal of Endocrinology*, **56**, 219–225.

Cavaille, F. and Maltier, J.P. (1978) A local signal from the gravid horn to the ovary for the onset of parturition in rats. *Journal of Reproduction and Fertility*, **54**, 227–231.

Challis, J.R. (1971) Sharp increase in free circulating oestrogens immediately before parturition in sheep. *Nature (London)*, **229**, 208.

Challis, J.R.G. and Linzell, J.L. (1971) The concentration of total unconjugated oestrogens in the plasma of pregnant goats. *Journal of Reproduction and Fertility*, **26**, 401–404.

Challis, J.R.G. and Illingworth, D.V. (1972) Oestrogens in the pregnant guinea-pig. *Journal of Reproduction and Fertility*, **31**, 485.

Challis, J.R.G. and Linzell, J.L. (1973) Oestrone metabolism in pregnant and lactating goats. *Journal of Endocrinology*, **57**, 451–457.

Challis, J.R.G. and Mitchell, B.F. (1988) Steroid production by the fetal membranes in relation to the onset of parturition, in, *The Onset of Labor: Cellular and Integrative Mechanisms*, (eds D. McNellis, J. Challis, P. MacDonald, P. Nathanielsz and J. Roberts), Perinatology Press, Ithaca, pp. 233–246.

Challis, J.R.G. and Olson, D.M. (1988) Parturition, in *The Physiology of Reproduction* (eds E. Knobil and J. Neill), Raven Press, New York, pp. 2177–2216.

Challis, J.R. and Patrick, J.E. (1981) Fetal and maternal estrogen concentrations thoughout pregnancy in the sheep. *Canadian Journal of Physiology and Pharmacology*, **59**, 970–978.

Challis, J.R.G. and Vaughan, M. (1987) Steroid synthetic and prostaglandin metabolising activity is present in different cell populations from human fetal membranes and decidua. *American Journal of Obstetrics and Gynecology*, **157**, 1474–1481.

Challis, J.R., Harrison, F.A. and Heap, R.B. (1971) Uterine production of oestrogens and progesterone at parturition in the sheep. *Journal of Reproduction and Fertility*, **25**, 306–307.

Challis, J.R., Harrison, F.A. and Heap, R.B. (1973a) The metabolic clearance rate, production rate and conversion ratios of oestrone in the sheep. *Journal of Endocrinology*, **58**, 435–446.

Challis, J.R.G., Davies, I.J. and Ryan, K.J. (1973b) The concentrations of progesterone, estrone and estradiol-17β in the plasma of pregnant rabbits. *Endocrinology*, **93**, 971–976.

Challis, J.R.G., Davies, I.J. and Ryan, K.J. (1973c) The relationship between progesterone and prostaglandin F concentrations in the plasma of pregnant rabbits. *Prostaglandins*, **4**, 509–516.

Challis, J.R.G., Davies, I.J., Benirschke, K., Hendrickx, A.G. and Ryan, K.J. (1974a) The effects of dexamethasone on plasma steroid levels and fetal adrenal histology in the pregnant rhesus monkey. *Endocrinology*, **95**, 1300–1305.

Challis, J.R.G., Davies, I.J., Benirschke, K., Henrickx, A.G. and Ryan, K.J. (1974b) The concentrations of progesterone, estrone and estradiol-17β in the peripheral plasma of the rhesus monkey during the final third of gestation, and after the induction of abortion with $PGF_{2\alpha}$. *Endocrinology*, **96**, 547–553.

Challis, J.R.G., Porter, D.G. and Ryan, K.J. (1974c) The effects of prostaglandin $F_{2\alpha}$ and ovariectomy on the peripheral plasma steroid concentrations and the evolution of myometrial activity in the pregnant rabbit. *Endocrinology*, **95**, 783–792.

Challis, J.R.G., Davies, I.J. and Ryan, K.J. (1975) The effect of dexamethasone and indomethacin on the outcome of pregnancy in the rabbit. *Journal of Endocrinology*, **64**, 363–370.

Challis, J.R.G., Dilley, S.R., Robinson, J.S. and Thorburn, G.D. (1976a) Prostaglandins in the circulation of the fetal lamb. *Prostaglandins*, **11**, 1041–1052.

Challis, J.R.G., Forster, C.S., Furr, B.J.A., Robinson, J.S. and Thorburn, G.D. (1976b) Production of prostaglandin F_2 in ewes following luteal regression induced with a prostaglan-

din analogue, Estrumate (Cloprostenol; ICI 80996). *Prostaglandins*, **11**, 537–541.

Challis, J.R., Hart, I., Louis, T.M., Mitchell, M.D., Jenkin, G., Robinson, J.S. and Thorburn, G.D. (1978) Prostaglandins in the sheep fetus, implications for fetal function. *Advances in Prostaglandin and Thromboxane Research*, **4**, 115–132.

Challis, J.R.G., Harrison, F.A., Heap, R.B., Horton, E.W. and Poyser, N.I. (1982) Possible role of oestrogens in the stimulation of $PGF_{2\alpha}$, output at the time of parturition in sheep. *Journal of Reproduction and Fertility*, **30**, 485–488.

Chambers, P.L. and Hearn, J.P. (1979) Peripheral plasma level of progesterone, oestradiol-17β, oestrone, testosterone, androstenedione and chorionic gonadotropin during pregnancy in the marmoset monkey, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, **56**, 23–32.

Chan, J.S.D., Robertson, H.A. and Friesen, H.G. (1978) Maternal and fetal concentrations of ovine placental lactogen measured by radioimmunoassay. *Endocrinology*, **102**, 1606–1613.

Chan, J.S., Grinwich, D.L., Robertson, H.A. and Friesen, H.G. (1980) Maintenance of receptors for luteinizing hormone by ovine placental lactogen in pseudopregnant rats. *Biology of Reproduction*, **23**, 60–63.

Chan, W.Y. (1987) Enhanced prostaglandin synthesis in the parturient rat uterus and its effects on myometrial oxytocin receptor concentrations. *Prostaglandins*, **34**, 889–902.

Chan, W.Y., Berezin, I. and Daniel, E.E. (1988) Effects of inhibition of prostaglandin synthesis on uterine oxytocin receptor concentration and myometrial gap junction density in parturient rats. *Biology of Reproduction*, **39**, 1117–1128.

Chantharaksri, U., Fuchs, A.R., Johansson, E.B.W. and Smitasiri, Y. (1974) Parturition and the effect of indomethacin in pregnant rats. *Gynecologic Investigation*, **5**, 30.

Cheah, S.H. and Sherwood, O.D. (1988) Effect of preparturient 17-beta-oestradiol and relaxin on parturition and pup survival in the rat. *Endocrinology*, **122**, 1958–1963.

Chernaeva, L. (1984) Adrenergic sensitivity of circular and longitudinal muscles of pregnant rat myometrium. *Methods and Findings in Experimental and Clinical Pharmacology*, **6**, 73–78.

Chew, P.C.T. and Ratnam, J. (1976) Serial levels of plasma oestradiol-17β at the approach of labour. *Journal of Endocrinology*, **71**, 267–268.

Chez, R.A., Hutchinson, D.L., Salazar, H. and Mintz, D.H. (1970) Some effects of fetal and maternal hypophysectomy in pregnancy. *American Journal of Obstetrics and Gynecology*, **108**, 643–649.

Chibbar, R., Miller, F.D. and Mitchell, B.F. (1991) Oxytocin is synthesized in human fetal membranes. *Society for Gynecologic Investigation, 38th Annual Meeting, San Antonio, Texas*, Abstract 7.

Chiboka, O., Casida, L.E. and First, N.L. (1976) Effect of fetectomy on pregnancy maintenance in swine (abstract). *Journal of Animal Science*, **42**, 1363.

Chiboka, O., Casida, L.E. and First, N.L. (1977) Role of rabbit fetuses and placentas in the maintenance of gestation and parturition. *Journal of Animal Science*, **45**, 776–783.

Choong, C.H. and Raeside, J.I. (1974) Chemical determination of oestrogen distribution in the foetus and placenta of the domestic pig. *Acta Endocrinologica*, **77**, 171–185.

Christie, G.A. (1968) Distribution of hydroxysteroid dehydrogenase in the placenta of the pig. *Journal of Endocrinology*, **40**, 285–291.

Chu, J.P., Lee, C.C. and You, S.S. (1946) Functional relation between the uterus and the corpora lutea. *Journal of Endocrinology*, **4**, 392–397.

Clabaut, M., Duclos, R. and Acritopolou-Fourcroy, S. (1988) Increase of uterine motility and simultaneous decrease of progesterone concentrations in the rat after bilateral ovariectomy at mid-pregnancy. *Journal of Reproduction and Fertility*, **82**, 545–551.

Clark, J.H., Hsueh, A.J.W. and Peck, E.J. (1977) Regulation of estrogen receptor levels by progesterone. *Annals of the New York Academy of Science*, **286**, 161–166.

Coggins, E.G. and First, N.L. (1973) Response of swine and rabbits to dexamethasone at different stages of gestation (abstract). *Journal of Animal Science*, **37**, 305.

Coggins, E.G. and First, N.L. (1977) Effect of dexamethasone, methallibure and fetal decapitation on porcine gestation. *Journal of Animal Science*, **44**, 1041–1049.

Coggins, E.G., Van Horn, D. and First, N.L. (1977) Influence of prostaglandin $F_{2\alpha}$, dexamethasone, progesterone and induced corpora lutea on porcine parturition. *Journal of Animal Science*, **46**, 754–762.

Cohen, D.K., Craig, D.A., Strickland, D.M., McCubbin, J.H. and Mitchell, M.D. (1985) Prostaglandin biosynthesis stimulatory and inhibitory substances in human amniotic fluid

during pregnancy and labour. *Prostaglandins*, **30**, 13–20.

Coleman, H.A. and Parkington, H.C. (1988) Induction of prolonged excitability in myometrium of pregnant guinea-pigs by prostaglandin $F_{2\alpha}$. *Journal of Physiology* **399**, 33–47.

Coleman, R.A., Humphrey, P.P.A. and Kennedy, I. (1985) Prostanoid receptors in smooth muscle, further evidence for a proposed classification, in, *Trends in Autonomic Pharmacology*,(eds S. Kalsner and F. Taylor) Vol. 3. London, pp. 35–49.

Comline, R.S., Hall, L.W., Lavelle, R.D., Nathanielsz, P.W. and Silver, M. (1974) Parturition in the cow, endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. *Journal of Endocrinology*, **63**, 451–456.

Concannon, P.W. and Hansel, W. (1977) Prostaglandin $F_{2\alpha}$-induced luteolysis, hypothermia and abortions in Beagle bitches. *Prostaglandins*, **13**, 533–542.

Concannon, P.W., Hansell, W. and Visek, W.J. (1975) The ovarian cycle of the bitch, Plasma oestrogen, LH and progesterone. *Biology of Reproduction*, **13**, 112–121.

Concannon, P.W., Powers, M.E., Holder, W. and Hansel, W. (1977) Pregnancy and parturition in the bitch. *Biology of Reproduction*, **16**, 517–526.

Concannon, P.W., Butler, W.R., Hansel, W., Knight, P.J. and Hamilton, J.M. (1978) Parturition and lactation in the bitch. Serum progesterone, cortisol and prolactin. *Biology of Reproduction*, **19**, 1113–1118.

Concannon, P.W., Isaman, L., Frank, D.A., Michel, F.J. and Currie, W.B. (1988) Elevated concentrations of 13,14-dihydro-14-keto-prosta glandin F_2 in maternal plasma during prepartum luteolysis and parturition in dogs (*Canis familiaris*). *Journal of Reproduction and Fertility*, **84**, 71–77.

Conley, A.J. and Ford, S.P. (1989) Direct luteotrophic effect of oestradiol-17β on pig corpora lutea. *Journal of Reproduction and Fertility*, **87**, 125–131.

Corner, G.W. (1928) Physiology of the corpus luteum. I. The effect of very early ablation of the corpus luteum upon embryo and uterus. *American Journal of Physiology*, **86**, 74–80.

Cornette, J.C., Harrison, K.L. and Kirton, K.T. (1974) Measurement of prostaglandin $F_{2\alpha}$ metabolites by radioimmunoassay. *Prostaglandins*, **5**, 155–164.

Cowie, A.T., Daniel, P.M., Prichard, M.M.L. and Tindal, J.S. (1963) Hypophysectomy in pregnant goats, and section of the pituitary stalk in pregnant goats and sheep. *Journal of Endocrinology*, **28**, 93–102.

Cox, R.I. (1975) The endocrinological changes of gestation and partuirtion in the sheep. *Advances in Veterinary Science and Comparative Medicine*, **19**, 287–305.

Craig, V.A. (1982) Placental steroid metabolism in late pregnancy, in *Control of Pig Reproduction*, (eds. D.J.A. Cole and G.R. Foxcroft), Butterworth Scientific, London, pp. 405–418.

Crankshaw, D.J. (1987) The sensitivity of the longitudinal and circular muscle layers of the rat's myometrium to oxytocin *in vitro* during pregnancy. *Canadian Journal of Physiology and Pharmacology*, **65**, 773–777.

Creange, J.E., Schane, H.P., Anzalone, A.J., Gordon, B.A. and Potts, O. (1978) Interruption of pregnancy in rats by azastene, an inhibitor of ovarian and adrenal steroidogenesis. *Fertility and Sterility*, **30**, 86–90.

Csapo, A.L. (1956) Progesterone block. *American Journal of Anatomy*, **98**, 273–291.

Csapo, A. (1969) The four direct regulatory factors of myometrial function, in, *Progesterone: Its Regulatory Effect on the Myometrium*, (eds G.E.W. Wolstenholme and J. Knight), Ciba Foundation Study Group No. 34, Churchill, London, pp. 13–42.

Csapo, A.I. and Pulkinnen, M.O. (1978) Indispensibility of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence. *Obstetrical and Gynecological Survey*, **33**, 69–81.

Csapo, A.I. and Weist, W.G. (1969) An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. *Endocrinology*, **35**, 735–746.

Csapo, A., Takeda, H. and Wood, C. (1963) Volume and activity of the parturient rabbit uterus. *American Journal of Obstetrics and Gynecology*, **85**, 813–818.

Csapo, A.I., De Souza Filho, M.B., De Souza, J.C. and De Souza, O. (1966) Effect of massive progestational hormone treatment on the parturient human uterus. *Fertility and Sterility*, **17**, 621–636.

Csapo, A.I., Dray, F. and Erdos, T. (1975) The biological effects of injected antibodies to estradiol-17β and to progesterone in pregnant rats. *Endocrinology*, **97**, 603–614.

Csapo, A.I., Puri, C.P. and Tarro, S. (1981)

Relationship between timing of ovariectomy and maintenance of pregnancy in the guinea pig. *Prostaglandins*, **22**, 131–140.

Cummings, S.W., Hatley, W., Simpson, E.R. and Ohashi, M. (1982) The binding of high and low density lipoproteins to human placental membrane fractions. *Journal of Clinical Endocrinology and Metabolism*, **54**, 903–908.

Currie, W.B. (1974) Foetal and maternal hormonal interactions controlling parturition in sheep and goats. *PhD Thesis*, Macquarie University, North Ryde, Australia.

Currie, W.B. and Thorburn, G.D. (1973) Induction of premature parturition in goats by prostaglandin $F_{2\alpha}$ administered into the uterine vein. *Prostaglandins*, **4**, 201–212.

Currie, W.B. and Thorburn, G.D. (1974) Luteal function in hysterectomized goats. *Journal of Reproduction and Fertility*, **41**, 501–504.

Currie, W.B. and Thorburn, G.D. (1976) Release of prostaglandin F, regression of corpora lutea and induction of premature parturition in goats treated with estradiol-17β. *Prostaglandins*, **12**, 1093–1103.

Currie, W.B. and Thorburn, G.D. (1977a) The fetal role in timing the initiation of parturition in the goat, in *The Fetus and Birth*, (eds. J. Knight and M. O'Connor), Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 46–66.

Currie, W.B. and Thorburn, G.D. (1977b) Parturition in goats, studies on the interactions between the foetus, placenta prostaglandin F and progesterone before parturition, at term or at parturition induced prematurely by corticotrophin infusion into the foetus. *Journal of Endocrinology*, **73**, 263–278.

Currie, W.B., Wong, M.S.F., Cox, R.I. and Thorburn, G.D. (1973) Spontaneous or dexamethasone induced parturition in the sheep and goat, changes in plasma concentrations of maternal prostaglandin F and foetal oestrogen sulphate. *Memoirs of the Society for Endocrinology*, **20**, 95–118.

Currie, W.B., Kelly, P.A., Friesen, H.G. and Thorburn, G.D. (1977) Caprine placental lactogen levels of prolactin-like and growth hormone-like activities in the circulation of pregnant goats determined by radioreceptor assay. *Journal of Endocrinology*, **73**, 215–226.

Dalle, M. and Delost, P. (1976) Plasma and adrenal cortisol concentrations in foetal, newborn and mother guinea pigs during the perinatal period. *Journal of Endocrinology*, **70**, 207–214.

Dalle, M. and Delost, P. (1980) Maturation of glucocorticoid activity in the foetal guinea pig during the end of gestation. *Reproduction, Nutrition and Development*, **20**, 331–337.

Daniel, E.E. and Singh, H. (1958) The electrical properties of the smooth muscle cell membrane. *Canadian Journal of Physiology and Pharmacology*, **36**, 959–966.

Dawood, M.Y., Ylikorkala, O., Trivedi, D. and Fuchs, F. (1979a) Oxytocin in maternal circulation and amniotic fluid during pregnancy. *Journal of Clinical Endocrinology and Metabolism*, **49**, 429–434.

Dawood, M.Y., Lauerson, N.H., Trivedi, D., Ylikorkala, O. and Fuchs, F. (1979b) Studies on oxytocin in the baboon during pregnancy and delivery. *Acta Endocrinologica*, **91**, 704–718.

Dawood, M.Y., Khan-Dawood, F.S., Ayromlooi, J. and Tobias, M. (1983) Maternal and fetal plasma oxytocin levels during pregnancy and parturition in the sheep. *American Journal of Obstetrics and Gynecology*, **147**, 584–588.

Debackere, M., Peeters, G. and Tuyttens, N. (1961) Reflex release of an oxytocic hormone by stimulation of genital organs in male and female sheep studied by a cross circulation technique. *Journal of Endocrinology*, **22**, 321–334.

De Cicco Nardone, F., D'Aurizio, G.M., Mori, P., Lupi, G., Vitale, A.M. and Dell'Aqua, S. (1984) Androgen binding in human fetal amnion. *Journal of Steroid Biochemistry*, **20**, 495–499.

Denamur, R. and Martinet, J. (1955) Effects de l'ovariectomie chez la brebis pendant la gestation. Societe de Biologie. *Comptes Rendus des Séances*, **149**, 2105–2107.

Denamur, R. and Martinet, J. (1961) Effect of hypophysectomy and pituitary stalk section on gestation in the sheep (in french). *Annales D'Endocrinologie*, **22**, 755–759.

Dhindsa, D.S. and Dziuk, P.J. (1968) Effect on pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation. *Journal of Animal Science*, **27**, 122–126.

DiRenzo, G.C., Johnston, J.M., Okazaki, T., Okita, J.R., MacDonald, P.C. and Bleasdale, J.E. (1981) Phosphatidylinositol-specific phospholipase C in fetal membranes and uterine decidua. *Journal of Clinical Investigation*, **67**, 847–856.

DiRenzo, G.C., Anceschi, M.M. and Bleasdale, J.E. (1984) Beta-adrenergic stimulation of prostaglandin production by human amnion tissue. *Prostaglandins*, **27**, 37–48.

Diskin, M.G., Wiltbank, M.C. and Niswender,

G.D. (1989) Inhibition of lipoprotein-stimulated steroidogenesis by prostaglandin $F_{2\alpha}$ is mediated through protein kinase C. *Journal of Reproduction and Fertility*, Abstract Series No. 3, Abstract 41.

Dolling, M. and Seamark, R.F. (1979) Progestagen metabolites in fetal sheep plasma: the effect of fetal nephrectomy. *Journal of Developmental Phsyiology*, **1**, 399–413.

Donaldson, L.E., Bassett, J.M. and Thorburn, G.D. (1970) Peripheral plasma progesterone concentrations of cows during puberty, oestrous cycles, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration. *Journal of Endocrinology*, **48**, 599–615.

Donovan, B.T. and Peddie, M.J. (1973) Foetal hypothalamic and pituitary lesions, the adrenal glands and abortion in the guinea pig, in, *Foetal and Neonatal Physiology*, (eds R.S. Comline, K.W. Cross, G.S. Dawes and P.W. Nathanielsz), Cambridge University Press, Cambridge, pp. 603–605.

Downing, S.J. and Porter, D.G. (1978) Evidence that inhibition of myometrial activity by oestradiol in the rat is mediated by an RNA synthetic pathway. *Journal of Endocrinology*, **78**, 119–124.

Downing, S.J. and Sherwood, O.D. (1985a) The physiological role of relaxin in the pregnant rat. I. The influence of relaxin on parturition. *Endocrinology*, **116**, 1200–1205.

Downing, S.J. and Sherwood, O.D. (1985b) The physiological role of relaxin in the pregnant rat. II. The influence of relaxin on uterine contractile activity. *Endocrinology*, **116**, 1206–1214.

Downing, S.J., Bradshaw, J.M.C. and Porter, D.G. (1980) Relaxin improves the coordination of rat myometrial activity *in vivo*. *Biology of Reproduction*, **23**, 899–903.

Dubin, N.H., Beck, J.C., Johnson, J.W.E., London, W.T., Ghodgaonkar, R.B. and Perlstein, M.J. (1988) Betamethasone effects on steroid and prostaglandin concentrations in pregnant rhesus monkeys. *American Journal of Obstetrics and Gynecology*, **159**, 233–237.

Ducsay, C.A., Cook, M.J., Walsh, S.W. and Novy, M.J. (1983) Circadian patterns and dexamethasone-induced changes in uterine activity in pregnant rhesus monkeys. *American Journal of Obstetrics and Gynecology*, **145**, 389–396.

Dufour, J. and Raeside, J.I. (1969) Hydroxysteroid dehydrogenase activity in the placenta of the domestic pig. *Endocrinology*, **84**, 426–431.

Dukes, M., Chester, R. and Atkinson, P. (1974) Effects of oestradiol and prostaglandin $F_{2\alpha}$ on the timing of parturition in the rat. *Journal of Reproduction and Fertility*, **38**, 325–334.

Dunn, M.V., Humphries, N.G., Judkins, G.R., Kendall, J.Z. and Knight, G.W. (1973) The effect of prostaglandin $F_{2\alpha}$ antibody on gestation length in the rat. *Prostaglandins*, **3**, 509–514.

Dusza, L. and Krzymowska, H. (1981) Plasma prolactin levels in sows during pregnancy, parturition and early lactation. *Journal of Reproduction and Fertility*, **61**, 131–134.

Dvorak, M. (1972) Adrenocortical function in foetal, neonatal and young pigs. *Journal of Endocrinology*, **54**, 473–481.

Dwyer, R.J. and Robertson, H.A. (1980) Oestrogen sulphatase and sulphotransferase activities in the endometrium of the sow and ewe during pregnancy. *Journal of Reproduction and Fertility*, **60**, 187–191.

Eddie, L.W., Lester, A., Bennet, G., Bell, R.J., Grier, M., Johnston, P.D. and Niall, H.D. (1986) Radioimmunoassay of relaxin in pregnancy with an analogue of human relaxin. *Lancet*, **1**, 1344–1347.

Edqvist, L.E., Ekman, L., Gustafsson, B. and Astrom, G. (1970) Progesterone levels in the bovine peripheral plasma measured by the competitive protein binding technique. *Zentralblatt fur Vetinarmedizin*, **17**, 899–905.

Edqvist, L.E., Ekman, L., Gustafsson, B., Jacobsson, S.O., Johansson, E.D.B. and Lindell, J.O. (1972) Peripheral plasma levels of oestrone and progesterone in pregnant cows treated with dexamethasone. *Acta Endocrinologica*, **71**, 731–742.

Edqvist, L.E., Ekman, L., Gustafsson, B. and Lindell, J.O. (1973a) Peripheral plasma levels of oestrone and progesterone after oophorectomy in the pregnant cow. *Acta Endocrinologica*, Suppl. 177, 43.

Edqvist, L.E., Ekman, L., Gustafsson, B. and Johansson, E.D.B. (1973b) Peripheral plasma levels of oestrogens and progesterone during late bovine pregnancy. *Acta Endocrinologica*, **72**, 81–88.

Edqvist, L.E., Kindahl, H. and Stabenfeldt, G. (1978) Release of prostaglandin F_2 alpha during the bovine peripartal period. *Prostaglandins*, **16**, 111–119.

Elger, W. (1979) Pharmacology of parturition and abortion. *Animal Reproduction Science*, **2**, 133–148.

Elger, W., Beier, S., Chwalisz, K., Fahnrich, M.,

Hasan, S.H., Henderson, D., Neef, G. and Rohde, R. (1986) Studies on the mechanism of action of progesterone antagonists. *Journal of Steroid Biochemistry*, **25**, 835–845.

Ellendorff, F., Forsling, M., Parvizi, N., Williams, H., Taverne, M. and Smidt, D. (1979) Plasma oxytocin and vasopressin concentrations in response to prostaglandin injections into the pig. *Journal of Reproduction and Fertility*, **56**, 573–577.

Ellicott, A.R. and Dziuk, P.J. (1973) Minimum daily dose of progesterone and plasma concentration for maintenance of pregnancy in ovariectomized gilts. *Biology of Reproduction*, **9**, 300–304.

Elsner, D.W., Magyar, D.M. and Fridshal, D. Eliot, J., Klein, A., Galtz, T., Nathanielsz, P.W. and Buster, J.E. (1980) Time-trend analysis of plasma C_{21} steroids in fetal and maternal sheep during the last 18 days of gestation. *Endocrinology*, **107**, 801–808.

Embrey, M.P. (1969) The effect of prostaglandins on human pregnant uterus. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **76**, 783–789.

Engstrom, T., Atke, A. and Vilhardt, H. (1988) Oxytocin receptors and contractile response of the myometrium after long term infusion of prostaglandin F_2 alpha, indomethacin, oxytocin and an oxytocin antagonist in rats. *Regulatory Peptides*, **20**, 65–72.

Estergreen, V.L., Frost, O.L., Gomes, W.R., Erb, R.E. and Bullard, J.F. (1967) Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. *Journal of Dairy Science*, **50**, 1293–1295.

Evans, C.A., Kennedy, T.G., Patrick, J.E. and Challis, J.R. (1981) Uterine prostaglandin concentrations in sheep during late pregnancy and adrenocorticotropin-induced labour. *Endocrinology*, **109**, 1533–1538.

Evans, C.A., Kennedy, T.G. and Challis, J.R. (1982) Gestational changes in prostanoid concentrations in intrauterine tissues and fetal fluids from pregnant sheep, and the relation to prostanoid output *in vitro*. *Biology of Reproduction*, **27**, 1–11.

Evans, G. and Wagner, W.C. (1981) *In vivo* oestrogen synthesis by bovine placenta during pregnancy and induced parturition. *Acta Endocrinologica*, **98**, 119–125.

Fairclough, R.J., Hunter, J.T. and Welch, R.A.S. (1975) Peripheral plasma progesterone and utero-ovarian prostaglandin F concentrations in the cow around parturition. *Prostaglandins*, **9**, 901–914.

Fairclough, R.J., Hunter, J.T., Welch, R.A.S., Barr, H. and Seamark, R.F. (1976) Preparturient changes in plasma concentrations of estrone, estradiol-17α and estradiol-17β in the fetal calf, effects of dexamethasone infusion. *Steroids*, **28**, 881–888.

Fairclough, R.J., Hunter, J.T., Peterson, A.J. and Welch, R.A.S. (1981) Plasma concentrations of progesterone, oestrogens and prostaglandin F in maternal blood and corticosteroids and oestrogens in fetal blood of cows during dexamethasone-induced deliveries. *Acta Endocrinologica*, **96**, 406–412.

Fairclough, R.J., Kaltenbach, C.C., Peterson, A.J., Welch, R.A.S., Cox, R.I. and Wong, M.S.F. (1984) Failure of exogenous progestagens to block dexamethasone-induced prostaglandin F release from the uterus of late pregnant cows. *Biology of Reproduction*, **30**, 112–118.

Ferguson, J.K.W. (1941) A study of the motility of the intact uterus at term. *Surgery in Gynecology and Obstetrics*, **73**, 359–366.

Fèvre, J. (1967) Urinary oestrogens in the ovariectomised pregnant ewe. *Annales de Biologie Animale, Biochimia, Biophysique*, **7**, 29.

Fèvre, J. and Rombauts, P. (1966) Étude de l'excrétion urinaire des oestrogènes chez la Brebis pendant la gestation. *Annales de Biologie Animale, Biochimie, Biophysique*, **6**, 165–177.

Fèvre, J., Lèglise, P.C. and Rombauts, P. (1968) Du rôle de l'hypophyse et des ovares dans la biosynthese des oestrogènes aux cours de la gestation chez la truie. *Annales de Biologie Animale, Biochimie, Biophysique*, **8**, 225–233.

Fields, M.J., Fields, P.A., Castro-Hernandez, A. and Larkin, L.H. (1980) Evidence for relaxin in corpora lutea of late pregnant cows. *Endocrinology*, **107**, 869–876.

Fields, P.A., Larkin, L.H. and Pardo, R.J. (1982) Purification of relaxin from the placenta of the rabbit. *Annals of the New York Academy of Sciences*, **380**, 75–86.

Findlay, J.K. and Cox, R.I. (1970) Oestrogens in the plasma of the sheep foetus. *Journal of Endocrinology*, **46**, 281–282.

Findlay, J.K. and Seamark, R.F. (1973) The occurrence and metabolism of oestrogens in the sheep foetus and placenta, in, *The Endocrinology of Pregnancy and Parturition*, (ed. C.G. Pierrepoint), Alpha Omega Alpha Publishing, Cardiff, UK, pp. 54–70.

First, N.L. (1972) Inhibition of parturition in swine by dithiocarbamoylhydrazine ICI 33828. *Journal of Reproduction and Fertility*, **30**, 321–323.

First, N.L. and Bosc, M.J. (1979) Proposed mechanisms controlling parturition and the induction of parturition in swine. *Journal of Animal Science*, **48**, 1407–1421.

First, N.L. and Staigmiller, R.B. (1973) Effects of ovariectomy, dexamethasone and progesterone on the maintenance of pregnancy in swine. *Journal of Animal Science*, **37**, 1191–1194.

First, N.L., Lohse, J.K. and Nara, B.S. (1982) The endocrine control of parturition, in, *Control of Pig Reproduction*, (eds D.J.A. Cole and G.R. Foxcroft), Butterworth Scientific, London, pp. 311–342.

Fitzpatrick, R.J. (1960) The reactivity of the ruminant uterus to posterior pituitary hormones. *Journal of Comparative Pathology and Therapeutics*, **70**, 36–58.

Fitzpatrick, R.J. and Walmsley, C.F. (1965) The release of oxytocin during parturition, in, *Advances in Oxytocin Research*, (ed. J.H.M. Pinkerton), Pergamon Press, Oxford, pp. 51–71.

Flint, A.P.F. (1979) Role of progesterone and oestrogens in the control of the onset of labour in Man: A continuing controversy, in, *Human Parturition*, (eds M.J.N.C. Keirse, A.B.M. Anderson and J.B. Gravenhorst), Leiden University Press, Leiden, pp. 85–100.

Flint, A.P.F. (1983) Regulation of placental enzymes. In, *Initiation of Parturition: Prevention of Prematurity*. Ross Conference, Dallas, USA, Ross Laboratories, pp. 27–34.

Flint, A.P.F., Anderson, A.B. and Turnbull, A.C. (1974a) Control of utero-ovarian and peripheral venous plasma progesterone by beta-sympathomimetic drugs in pregnant sheep. *Journal of Endocrinology*, **63**, 253–254.

Flint, A.P.F., Anderson, A.B., Patten, P.T. and Turnbull, A.C. (1974b) Control of utero-ovarian venous prostaglandin F during labour in the sheep: acute effects of vaginal and cervical stimulation. *Journal of Endocrinology*, **63**, 67–87.

Flint, A.P.F., Forsling, M.C., Mitchell, M.D. and Turnbull, A.C. (1975) Temporal relationship between changes in oxytocin and PGF levels in response to vaginal distension in the pregnant and puerperal ewe. *Journal of Reproduction and Fertility*, **43**, 551–554.

Flint, A.P.F., Kingston, E.J., Robinson, J.S. and Thorburn, G.D. (1978) The initiation of parturition in the goat: evidence for control by fetal glucocorticoid through activation of placental C_{21}-steroid 17α-hydroxylase. *Journal of Endocrinology*, **78**, 367–378.

Flint, A.P.F., Ricketts, A.P. and Craig, V.A. (1979) The control of placental steroid synthesis in domestic animals. *Animal Reproductive Science*, **3**, 239–251.

Ford, S.P., Magness, R.R., Farley, D.B. and Van Orden, D.E. (1982) Local and systemic effects of intra-uterine oestradiol-17β on luteal function of nonpregnant sows. *Journal of Animal Science*, **55**, 657–664.

Forsling, M.L., Taverne, M.A.M., Parvizi, N., Elsaesser, F., Smidt, D. and Ellendorff, F. (1979) Plasma oxytocin and steroid concentrations during late pregnancy, parturition and lactation in the miniature pig. *Journal of Endocrinology*, **82**, 61–69.

Fowden, A.L. and Silver, M. (1983) The effect of nutritional state on uterine prostaglandin F metabolite concentrations in the pregnant ewe during late gestation. *Quarterly Journal of Experimental Physiology*, **68**, 337–349.

Fowden, A.L. and Silver, M. (1987) Effects of inhibiting 3β-hydroxysteroid dehydrogenase on plasma progesterone and other steroids in the pregnant mare near term. *Journal of Reproduction and Fertility*, Suppl. 35, 539–545.

Fowden, A.L., Harding, R., Ralph, M.M. and Thorburn, G.D. (1987) The nutritional regulation of plasma prostaglandin E concentrations in the fetus and pregnant ewe during late gestation. *Journal of Physiology (London)*, **394**, 1–12.

Fraenkel, L. (1910) Die Funktion des Corpus Luteum. *Archiv fur Gynaekologie*, **68**, 438–462.

France, J.T. and Liggins, G.C. (1969) Placental sulphatase deficiency. *Journal of Clinical Endocrinology and Metabolism*, **29**, 138–141.

France, J.T., Mason, J.I., Magness, R.R., Murry, B.A. and Rosenfeld, C.R. (1987) Ovine placental aromatase: studies of activity levels, kinetic characteristics and effects of aromatase inhibitors. *Journal of Steroid Biochemistry*, **28**, 155–160.

France, J.T., Magness, R.R., Murry, B.A., Rosenfeld, C.R. and Mason, J.I. (1988) The regulation of ovine placental steroid 17 alpha-hydroxylase and aromatase by glucocorticoid. Molecular *Endocrinology*, **2**, 193–199.

Frank, M., Bazer, F.W., Thatcher, W.W. and Wilcox, C.J. (1977) A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine. III. Effects of estradiol valerate on prostaglandin $F_{2\alpha}$, progestins, estrone and estradiol concentrations in the

utero-ovarian vein of nonpregnant gilts. *Prostaglandins*, **14**, 1183–1196.

Fuchs, A.R. (1964) Oxytocin and the onset of labour in rabbits. *Journal of Endocrinology*, **30**, 217–227.

Fuchs, A.R. (1969) Uterine activity in late pregnancy and during parturition in the rat. *Biology of Reproduction*, **1**, 344–353.

Fuchs, A.R. (1978) Hormonal control of myometrial function during pregnancy and parturition. *Acta Endocrinologica*, Suppl. 221, 9–70.

Fuchs, A.R. and Poblete, V.F. (1970) Oxytocin and uterine function in pregnant and parturient rats. *Biology of Reproduction*, **2**, 387–400.

Fuchs, A.R., Mok, E. and Sundaram, K. (1974) Luteolytic effects of prostaglandins in rat pregnancy, and reversal by luteinizing hormone. *Acta Endocrinologica*, **76**, 583–596.

Fuchs, A.R., Smitasiri, Y. and Chantharaksri, U. (1976) The effect of indomethacin on uterine contractility and luteal regression in pregnant rats at term. *Journal of Reproduction and Fertility*, **48**, 331–340.

Fuchs, A.R., Husslein, P. and Fuchs, F. (1981) Oxytocin and the initiation of labour. II. Stimulation of prostaglandin production in human decidua by oxytocin. *American Journal of Obstetrics and Gynecology*, **141**, 694–697.

Fuchs, A.R., Fuchs, F., Husslein, P., Soloff, M.S. and Fernstrom, M. (1982a) Oxytocin receptors and human parturition, A dual role for oxytocin in the initiation of labor. *Science*, 215,1369-1398.

Fuchs, A.R., Husslein, P., Sumulong, L. and Fuchs, F. (1982b) The origin of circulating 13,14-dihydro-15-keto-$PGF_{2\alpha}$ during delivery. *Prostaglandins*, **24**, 715–722.

Fuchs, A.R., Goeschem, K., Husslein, P., Rasmussen, A.B. and Fuchs, F. (1983a) Oxytocin and the initiation of human parturition. III. Plasma concentrations of oxytocin and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in spontaneous and oxytocin induced labor at term. *American Journal of Obstetrics and Gynecology*, **147**, 497–502.

Fuchs, A.R., Periyasamy, S., Alexandrova, M. and Soloff, M.S. (1983b) Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology*, **113**, 742–749.

Fuchs, A.R., Fuchs, F., Husslein, P. and Soloff, M.S. (1984) Oxytocin receptors in the human uterus during pregnancy and parturition. *American Journal of Obstetrics and Gynecology*, **150**, 734–741.

Fylling, P. (1970) The effect of pregnancy, ovariectomy and parturition on plasma progesterone level in sheep. *Acta Endocrinologica*, **65**, 273–283.

Gadsby, J.E., Keyes, P.L. and Bill, C.H. (1983) Control of corpus luteum function in the pregnant rabbit: role of estrogen and lack of a direct luteotropic role of the placenta. *Endocrinology*, **113**, 2255–2261.

Gardner, M.L., First, N.L. and Casida, L.E. (1963) Effect of exogenous estrogens on corpus luteum maintenance in gilts. *Journal of Animal Science*, **22**, 132–134.

Garfield, R.E. (1988) Structural and functional studies of the control of myometrial contractility and labor, in, *The Onset of Labor: Cellular and Integrative Mechanisms*, (eds D. McNellis, J. Challis, P. MacDonald, P. Nathanielsz and J. Roberts), Perinatology Press, Ithaca, pp. 55–80.

Garfield, R.E., Hayashi, R.H. and Harper, M.J. (1987) *In vitro* studies on the control of human myometrial gap junctions. *International Journal of Gynaecology and Obstetrics*, **25**, 241–248.

Garverick, H.A., Polge, C. and Flint, A.P.F. (1982) Oestradiol administration raises luteal LH receptor levels in intact and hysterectomized pigs. *Journal of Reproduction and Fertility*, **66**, 371–377.

Gibb, W., Lavoie, J.C. and Roux, J.F. (1978) *In vitro* conversion of pregnenolone to progesterone by term human fetal membranes. *American Journal of Obstetrics and Gynecology*, **136**, 631–634.

Gibbens, D., Boyd, N.R.H. and Chard, T. (1972) Spurt release of oxytocin during human labour. *Journal of Endocrinology*, **53**, 1iv–1v.

Gibori, G., Keyes, P.L. and Richards, J.S. (1978) A role for intraluteal estrogen in the mediation of luteinizing hormone action on the rat corpus luteum during pregnancy. *Endocrinology*, **103**, 162–169.

Gibori, G., Basuray, R. and McReynolds, B. (1981) Luteotropic role of the decidual tissue in the rat, dependency on intraluteal estradiol. *Endocrinology*, **108**, 2060–2066.

Gillespie, A. (1973) Prostaglandins and human labour, in *Endocrine Factors in Labour*, (eds A. Klopper and J. Gardner) Cambridge University Press, Cambridge, pp. 77–93.

Gimenez, T., Louis, T.M. and Henricks, D.M. (1983) Fetal–maternal secretion of prostaglandins in the cow. *Prostaglandins*, **25**, 511–518.

Gimeno, A.L., Chaud, M., Bonacossa, A., Franchi, A.M. and Gimeno, M.F. (1983)

Prostacyclin induces a surprising long-lasting motility in quiescent uterine strips (indomethacin-treated) isolated from ovariectomized rats. *Prostaglandins*, **26**, 663–676.

Ginther, O.J. (1979) *Reproductive Biology of the Mare*. Ginther, Asas Plains, Wisconsin.

Glatz, T.H., Weitzman, R.E., Eliot, R.J., Klein, A.H., Nathanielsz, P.W. and Fisher, D.A. (1981) Ovine maternal and fetal plasma oxytocin concentrations before and during parturition. *Endocrinology*, **108**, 1328–1332.

Gleeson, A.R., Thorburn, G.D. and Cox, R.I. (1974) Prostaglandin F concentrations in the utero-ovarian venous plasma of the sow during the late luteal phase of the oestrous cycle. *Prostaglandins*, **5**, 521–529.

Glickman, J.A., Carson, G.D. and Challis, J.R.G. (1979) Differential effects of synthetic adrenocorticotrophin and α-melanocyte-stimulating hormone on adrenal function in human and sheep fetuses. *Endocrinology*, **104**, 34–39.

Goland, R.S., Wardlaw, S.L., Stark, R.I., Brown, L.S. and Frantz, A.G. (1986) High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy and parturition. *Journal of Clinical Endocrinology and Metabolism*, **63**, 1199–1203.

Golander, A., Barrett, J., Hurley, J., Barry, S. and Handwerger, S. (1979) Failure of bromocriptine, dopamine, and thyrotropin releasing hormone to affect prolactin secretion by human decidual tissue *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **49**, 787–789

Goldenberg, R.L., Bridson, W.E. and Kohler, P.O. (1972) Estrogen stimulation of progesterone synthesis by porcine granulosa cells in culture. *Biochemical and Biophysical Research Communications*, **48**, 101–107.

Gomes, W.R., Frost, O.L. and Estergreen, V.L. (1962) Progestin in ovarian and peripheral blood of cows during late pregnancy. *Journal of Dairy Science*, **45**, 670–675.

Gordon, W.L. and Sherwood, O.D. (1983) Evidence for a role of prostaglandins in the antepartum release of relaxin in the pregnant rat. *Biology of Reproduction*, **28**, 154–160.

Gorodeski, G.I., Sheehan, L.A. and Utian, W.H. (1990) Myometrial oxytocin receptor levels in the pregnant rat are higher in distal than in proximal portions of the horn and correlate with disparate oxytocin responsive myometrial contractility in these segments. *Endocrinology*, **127**, 1136–1143.

Grandis, A., Jorgensen, V., Kodack, L., Quarfordt, S. and Handwerger, S. (1989) High-density lipoproteins (HDL) stimulate placental lactogen secretion in pregnant ewes, further evidence for a role of HDL in placental lactogen secretion during pregnancy. *Journal of Endocrinology*, **120**, 423–427.

Greén, K., Bygdeman, M., Toppozada, M. and Wiqvist, N. (1974) The role of prostaglandin $F_{2\alpha}$ in human parturition. Endogenous plasma levels of 15-keto-13,14-dihydro-prostaglandin F_2 during labor. *American Journal of Obstetrics and Gynecology*, **120**, 25–31.

Gross, T.S. and Williams, W.F. (1988) Bovine placental prostaglandin synthesis: principal cell synthesis as modulated by the binucleate cell. *Biology of Reproduction*, **38**, 1027–1034.

Gross, T.S., Williams, W.F., Manspeaker, J.E., Lewis, G.S. and Russek-Cohen, E. (1987) Bovine placental prostaglandin synthesis *in vitro* as it relates to placental separation. *Prostaglandins*, **34**, 903–917.

Gu, W. and Rice, G.E. (1991) Arachidonic acid metabolites in pregnant rat uterus. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **42**, 15–18.

Guico-Lamm, M.L. and Sherwood, O.D. (1988) Monoclonal antibodies specific for rat relaxin. II. Passive immunization with monoclonal antibodies throughout the second half of pregnancy disrupts birth in intact rats. *Endocrinology*, **123**, 2479–2485.

Gustavii, B. and Greén, K. (1972) Release of prostaglandin $F_{2\alpha}$ following injection of hypertonic saline for therapeutic abortion: A preliminary study. *American Journal of Obstetrics and Gynecology*, **114**, 1099–1100.

Hagen, D.R., Houtz, S.K., Griel, L.C. and Kavanaugh, J.F. (1983) Concentrations of progesterone in arterial and venous plasma of fetal pigs and their dams in late gestation. *Biology of Reproduction*, **29**, 919–923.

Haluska, G.J. and Currie, W.B. (1988) Variation in plasma concentrations of oestradiol-17β and their relationship to those of progesterone, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ and oxytocin across pregnancy and at parturition in pony mares. *Journal of Reproduction and Fertility*, **84**, 635–646.

Haluska, G.J., Stanezyk, F.Z., Cook, M.J. and Novy, M.J. (1987a) Temporal changes in uterine activity and prostaglandin response to RU486 in rhesus macaques in late gestation. *American*

Journal of Obstetrics and Gynecology, **157**, 1487–1495.

Haluska, G.J., Lowe, J.E. and Currie, W.B. (1987b) Electromyographic properties of the myometrium correlated with the endocrinology of the prepartum and postpartum periods and parturition in pony mares. *Journal of Reproduction and Fertility*, **35**, 553–564.

Haluska, G.J., West, N.B., Novy, M.J. and Brenner, R.M. (1990a) Uterine estrogen receptors are increased by RU486 in late pregnant rhesus macaques but not after spontaneous labor. *Journal of Clinical Endocrinology and Metabolism*, **70**, 181–186.

Haluska, G.J., Mitchell, M.D. and Novy, M.J. (1990b) Amniotic fluid lipoxygenase metabolites during spontaneous labor and after RU486 treatment during late pregnancy in rhesus macaques. *Prostaglandins*, **40**, 99–105.

Hanahan, D.J. (1986) Platelet activating factor: A biologically active phosphogylceride. *Annual Review of Biochemistry*, **55**, 483–509.

Hancock, J.L., McGovern, P.T. and Stamp, J.T. (1968) Failure of gestation of goat [40]X sheep hybrids in goats and sheep. *Journal of Reproduction and Fertility*, Suppl. 3, 29–36.

Handwerger, S., Barry, S., Barrett, J., Markoff, E., Zeitler, P., Cwikel, B. and Siegel, M. (1981) Inhibition of the synthesis and secretion of decidual prolactin by arachidonic acid. *Endocrinology*, **109**, 2016–2021.

Handwerger, S., Quarfordt, S., Barrett, J. and Harman, I. (1987) Apolipoproteins AI, AII and CI stimulate placental lactogen release from human placental tissue. A novel action of high density lipoprotein apolipoproteins. *Journal of Clinical Investigation*, **79**, 625–628.

Hartikainen-Sorri, A.L., Kauppila, A., Tuimala, R., Viinikka, L. and Ylikorkala, O. (1981) The lack of significant change in plasma progesterone and estradiol-17β before the onset of human labor. *Acta Obstetrica Gynecologica Scandinavica*, **60**, 497–499.

Haukkamaa, M. and Lahteenmaki, P. (1979) Steroids in human myometrium and maternal and umbilical cord plasma before and during labor. *Obstetrics and Gynecology*, **53**, 617–622.

Heap, R.B. and Illingworth, D.V. (1974) The maintenance of gestation in the guinea pig and other hystricomorph rodents: changes in the dynamics of progesterone metabolism and the occurence of progesterone-binding globulin (PBG). *Symposia of the Zoological Society (London)*, **34**, 385–415.

Heap, R.B., Galil, A.K.A., Harrison, F.A., Jenkin, G. and Perry, J.S. (1977) Progesterone and oestrogen in pregnancy and parturition, comparative aspects and hierarchical control, in *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 127–157.

Heap, R.B., Flint, A.P.F., Hartman, P.E., Gadsby, J.E., Staples, L.D., Ackland, N. and Hamon, M. (1981) Oestrogen production in early pregnancy. *Journal of Endocrinology*, **89**, 77P–94P.

van Heerden, K.M. (1961) Investigation into the cause of abortion in angora goats in South Africa. *Journal of the South African Veterinary Medical Association*, **32**, 211.

Heinricks, W.L. and Colas, A.E. (1970) Hepatic microsomal 16-hydroxylation of 3β-hydroxyandrost-5-en-17-one (DHA) by fetal, newborn and adult rhesus monkeys. *General and Comparative Endocrinology*, **14**, 159–163.

Hellig, H., Gattereau, D., Lefebvre, Y. and Bolt, E. (1970) Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to placental progesterone in humans. *Journal of Clinical Endocrinology and Metabolism*, **30**, 624–631.

Hempel, R. and Newmann, F. (1965) Hemming der Uteruswirkung von Oxytocin durch wasserlosliche steroide. *Acta Endocrinologica*, **48**, 656–663.

Henderson, K.M., Scaramuzzi, R.J. and Baird, D.T. (1977) Simultaneous infusion of prostaglandin E_2 antagonizes the luteolytic action of prostaglandin $F_{2\alpha}$ *in vivo*. *Journal of Endocrinology*, **73**, 379–383.

Higuchi, T., Honda, K., Fukuoka, T., Negoro, H. and Wakabayashi, K. (1985a) Release of oxytocin during suckling and parturition in the rat. *Journal of Endocrinology*, **105**, 339–346.

Higuchi, T., Ulchide, K., Honda, K. and Negoro, H. (1985b) Functional development of the oxytocin release mechanism and its role in the initiation of parturition in the rat. *Journal of Endocrinology*, **106**, 311–316.

Higuchi, T., Uchida, K., Honda, K. and Negoro, H. (1986) Oxytocin release during parturition in the pelvic neurectomized rat. *Endocrinology*, **109**, 149–154.

Hilary, H.K. and Cohen, S.L. (1981) The changing oestrogen content of the nuclear fraction of hu-

man myometrium during labor. *Steroids*, **38**, 175–184.

Hillman, R.B. (1975) Induction of parturition in mares. *Journal of Reproduction and Fertility*, **23**, 641–644.

Hindson, J.C., Schofield, B.M. and Turner, C.B. (1967) The effect of a single dose of stilbestrol on cervical dilation in pregnant sheep. *Research in Veterinary Science*, **8**, 353–360.

Hindson, J.C., Schofield, B.M. and Ward, W.R. (1969) The effect of progesterone on recorded parturition and on oxytocin sensitivity in the sheep. *Journal of Endocrinology*, **43**, 207–215.

Hirata, F. (1983) Lipomodulin: A possible mediator of the action of glucocorticoids, in *Advances in Prostaglandin, Leukotriene and Thromboxane Research*, (eds B. Samuelsson, R. Paoletti and P. Ramwell), Raven Press, New York, pp. 73–78.

Hirata, F., Schiffmann, K., Venkatasubramanian, K., Salomon, D. and Axelrod, J. (1980) Phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proceedings of the National Academy of Science of the USA*, **77**, 2533–2536.

Hirst, J.J., Haluska, G.J., Cook, M.J. and Novy, M.J. (1991) Maternal plasma oxytocin and oxytocin receptor concentrations during late pregnancy in rhesus monkeys. *Society for Gynecologic Investigation*, Abstract 95.

Hixon, J.E. and Flint, A.P.F. (1987) Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2 alpha secretion in sheep. *Journal of Reproduction and Fertility*, **79**, 457–467.

Hodgen, G.D., Stouffer, R.L., Barber, D.L. and Nixon, W.E. (1977) Serum estradiol and progesterone during pregnancy and the status of the corpus luteum at delivery in cynomolgus monkeys (*Macaca fascicularis*). *Steroids*, **30**, 295–301.

Hodges, J.K., Henderson, C. and Hearn, J.P. (1983) Relationship between ovarian and placental steroid production during early pregnancy in the marmoset monkey (*Callithrix jacchus*). *Journal of Reproduction and Fertility*, **69**, 613–621.

Hoedemaker, M., Weston, P. and Wagner, W.C. (1990) Influence of cortisol and different steroidogenic pathways on estrogen synthesis by the bovine placenta. *American Journal of Veterinary Research*, **51**, 1012–1015.

Hoffman, B., Schams, D., Gimenez, T., Ender, M.L., Herrmann, C.H. and Karg, H. (1973) Changes of progesterone, total oestrogens, corticosteroids, prolactin and LH in bovine peripheral plasma around parturition with special reference to the effect of exogenous corticoid and prolactin inhibitor respectively. *Acta Endocrinologica*, **73**, 385–395.

Hoffman, B., Wagner, W.C., Rattenberger, E. and Schmidt, J. (1977) Endocrine relationships during late gestation and parturition in the cow, in *The Fetus and Birth*, (eds J. Knight and M. O'Connor) Ciba Symposium No. 47, Elsevier, New York, pp. 107–118.

Hoffman, B., Wagner, W.C., Hixon, J.E. and Bahr, J. (1979) Determination concerning the functional status of the corpus luteum and the placenta around parturition in the cow. *Animal Reproductive Science*, **2**, 253–266.

Hoffman, D.R., Truong, T.C. and Johnston, J.M. (1986) The role of platelet-activating factor in human fetal lung maturation. *American Journal of Obstetrics and Gynecology*, **155**, 70–75.

Holm, L.W. (1967) Prolonged pregnancy. *Advances in Veterinary Science*, **11**, 159–205.

Holtan, D.W., Nett, T.M. and Estergren, V.L. (1975) Plasma progestagens in pregnant mares. *Journal of Reproduction and Fertility*, Suppl. 23, 419–424.

Holtan, D.W., Squires, E.L., Lapin, D.R. and Ginther, O.J. (1979) Effect of ovariectomy on pregnant mares. *Journal of Reproduction and Fertility*, Suppl. 27, 457–463.

Holtan, D.W., Houghton, E., Silver, M., Fowden, A.L., Ousey, J. and Rossdale, P.D. (1991) Plasma progestagens in the mare, fetus and newborn foal. *Journal of Reproduction and Fertility*, Suppl. 44, 517–528.

Honnebier, M.B.O.M., Figueroa, J.P., Rivier, J., Vale, W. and Nathanielsz, P.W. (1989a) Studies on the role of oxytocin in late pregnancy in the pregnant rhesus monkey: plasma concentrations of oxytocin in the maternal circulation throughout the 24h day and the effect of the synthetic oxytocin antagonist [^{1}β-Mpa (β-$(CH_2)_5$)1, (Me)Tyr^2, Orn^8] Oxytocin on spontaneous nocturnal myometrial contractions. *Journal of Developmental Physiology*, **12**, 225–232.

Honnebier, M.B.O.M., Myers, T., Figueroa, J.P. and Nathanielsz, P.W. (1989b) Variation in myometrial response to intravenous oxytocin administration at different times of the day in the pregnant rhesus monkey. *Endocrinology*, **125**, 1498–1503.

Honnebier, W.M. and Swaab, D.F. (1973) The influence of anencephaly upon intrauterine growth of the fetus and placenta and upon gestation length. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 577–588.

Hsueh, A.J., Peck, E.J. Jr. and Clark, J.H. (1976) Control of uterine estrogen receptor levels by progesterone. *Endocrinology*, **98**, 438–444.

Hudson, P., Haley, J., John, M., Cronk, M., Crawford, R., Haralambidis, J., Tregear, G., Shine, J. and Niall, H. (1983) Structure of a genomic clone encoding biologically active human relaxin. *Nature*, **301**, 628–631.

Hunter, J.T., Welch, R.A.S., Fairclough, R.J., Barr, H. and Seamark, R.F. (1974) Foetal oestrogens and maternal progestins when the foetus was infused with dexamethasone. *Journal of Reproduction and Fertility*, **36**, 469–470.

Hunter, J.T., Fairclough, R.J., Peterson, A.J. and Welch, R.A.S. (1977) Foetal and maternal hormonal changes preceding normal bovine parturition. *Acta Endocrinologica*, **84**, 653–662.

Husslein, P. (1984) Concentration of 13,14-dihydro-15-keto-prostaglandin E_2 in the maternal peripheral plasma during labour of spontaneous onset. *British Journal of Obstetrics and Gynaecology*, **91**, 228–231.

Hwang, J.J. and Sherwood, O.D. (1988) Monoclonal antibodies specific for rat relaxin. III. Passive immunization with monoclonal antibodies throughout the second half of pregnancy. *Endocrinology*, **123**, 2486–2490.

Illingworth, D.V., Challis, J.R.G., Ackland, N., Burton, A.M., Heap, R.B. and Perry, J.S. (1974) Parturition in the guinea pig; plasma levels of steroid hormones, steroid-binding proteins, and oxytocin and the effect of corticosteroids, prostaglandins and adrenocorticotrophin. *Journal of Endocrinology*, **63**, 557–570.

Irving, G., Jones, D.E. and Knifton, A. (1972a) Progesterone concentrations in the peripheral plasma of pregnant goats. *Journal of Endocrinology*, **53**, 447–452.

Irving, G., Jones, D.E. and Knifton, A. (1972b) Progesterone concentration in the peripheral plasma of pregnant goats after ovariectomy and replacement therapy. *Research in Veterinary Science*, **13**, 301–303.

Jackson, J.A. and Albrecht, E.D. (1985) The development of placental androstenedione and testosterone production and their utilization by the ovary for aromatization to estrogen during rat pregnancy. *Biology of Reproduction*, **33**, 451–458.

Janigan, D.T., Smith, O.D. and Nichols, J. (1962) Observations on the central nervous system, pituitary and adrenal in two cases of microcephaly. *Journal of Clinical Endocrinology and Metabolism*, **22**, 683–687.

Jarvinen, P.A., Luukkainen, T. and Vaisto, L. (1965) The effect of estradiol infusion on uterine activity in late pregnancy. *Acta Obstetrica Gynecologica Scandinavica*, **44**, 258–264.

Jasper, D.E. (1950) Prolonged gestation in the bovine. *Cornell Veterinarian*, **40**, 165–172.

Jenkin, G. and Thorburn, G.D. (1985) Inhibition of progesterone secretion by a 3 beta-hydroxysteroid dehydrogenase inhibitor in late pregnant sheep. *Canadian Journal of Physiology and Pharmacology*, **63**, 136–142.

Jenkin, G., Jorgensen, G., Young, I.R. and Thorburn, G.D. (1982) Prostaglandin $F_{2\alpha}$ and parturition in the sheep. *Australian Society for Reproductive Biology*, **14**, Abstract 31.

Jenkin, G., Jorgensen, G., Thorburn, G.D., Nathanielsz, P.W. and Buster, J.E. (1985a) Progesterone metabolism during induction of delivery by infusion of cortisol to the ovine fetus, in, *The Physiological Development of the Fetus and Newborn*, (eds C.T. Jones and P.W. Nathanielsz), Academic Press, London, pp. 427–432.

Jenkin, G., Jorgensen, G., Thorburn, G.D., Buster, J.E. and Nathanielsz, P.W. (1985b) Induction of premature delivery in sheep following infusion of cortisol to the fetus. I. The effect of maternal administration of progestagens. *Canadian Journal of Physiology and Pharmacology*, **63**, 500–508.

Jenkin, G., Burgess, K.M., Ralph, M.M., Harding, R. and Thorburn, G.D. (1989) Prostaglandin release in response to oxytocin in mother and fetus in late ovine pregnancy. *Journal of Reproduction and Fertility*, Abstract series No. 4, Abstract 19.

Jenkin, G., Brouwer, D., Harding, R. and Melin, P. (1990) The mechanism of action of the oxytocin inhibitor CAP 449 in late pregnant sheep. *The Endocrine Society of Australia, 33rd Annual Meeting*, Perth, Abstract 45.

Johansson, E.D.B. (1971) Depression of the progesterone levels in women treated with synthetic gestagens after ovulation. *Acta Endocrinologica*, **68**, 779–792.

Jones, S.A. and Summerlee, A.J. (1986a) Effects of

porcine relaxin on the length of gestation and duration of parturition in the rat. *Journal of Endocrinology*, **109**, 85–88.

Jones, S.A. and Summerlee, A.J. (1986b) Relaxin acts centrally to inhibit oxytocin release during parturition, an effect that is reversed by naloxone. *Journal of Endocrinology*, **111**, 99–102.

Jung, H. (1962) Electrophysiology of the uterus and electrohysterography. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **69**, 1040–1044.

Kao, C.Y. and Nishiyama, A. (1964) Ovarian hormones and resting potential of rabbit smooth muscle. *American Journal of Physiology*, **207**, 793–798.

Kawano, M. and Mori, N. (1988) The significance of prostacyclin produced by pregnant rat myometrium. *Prostaglandins*, **35**, 305–325.

Keirse, M.J.N.C. (1979) Endogenous prostaglandins in human parturition, in *Human Parturition*, (eds. M.J.N.C. Keirse, A.B.M. Anderson and J.B. Gravenhorst), Leiden University Press, Leiden, pp. 101–142.

Keirse, M.J.N.C. and Turnbull, A.C. (1972) E prostaglandins in amniotic fluid during late pregnancy and labour. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 131–135.

Keirse, M.J.N.C., Flint, A.P.F. and Turnbull, A.C. (1974) F prostaglandins in amniotic fluid during pregnancy and labour. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 131–135.

Keirse, M.J., Hicks, B.R. and Turnbull, A.C. (1976) Metabolism of prostaglandin F_2 alpha in fetal and maternal cotyledons of sheep. *Journal of Reproduction and Fertility*, **46**, 417–420.

Keirse, M.J.N.C., Hicks, B.R., Mitchell, M.D. and Turnbull, A.C. (1977) Increase of the prostaglandin precursor, arachidonic acid, in amniotic fluid during spontaneous labour. *British Journal of Obstetrics and Gynaecology*, **84**, 937–940.

Keirse, M.J.N.C., Moonen, P. and Klok, G. (1984) Prostaglandin synthase in pregnant human myometrium is not confined to the uteroplacental vasculature. *IRCS Medical Science*, **12**, 824–825.

Kelly, P.A., Tusuhima, T., Shiu, R.P.C. and Friesen, H.G. (1976) Lactogenic and growth hormone-like activities during pregnancy determined by radioreceptor assays. *Endocrinology*, **99**, 765–774.

Kelly, R.W. and Smith, R.K. (1987) Progesterone and antiprogestins, a comparison of their effect on prostaglandin production by human secretory phase endometrium and decidua. *Prostaglandins, Leukotrienes and Medicine*, **29**, 181–186.

Kelly, R.W., Deam, S., Cameron, M.J. and Seamark, R.F. (1986) Measurement by radioimmunoassay of prostaglandins as their methyl oximes. *Prostaglandins, Leukotrienes and Medicine*, **24**, 1–14.

Kendall, J.Z. (1977) *PhD Thesis*. University of Auckland, New Zealand.

Kendall, J.Z. and Liggins, G.C. (1972) The effect of dexamethasone on pregnancy in the rabbit. *Journal of Reproduction and Fertility*, **29**, 409–413.

Kendall, J.Z., Challis, J.R.G., Hart, I.C., Jones, C.T., Mitchell, M.D., Ritchie, J.W.K., Robinson, J.S. and Thorburn, G.D. (1977) Steroid and prostaglandin concentrations in the plasma of pregnant ewes during infusion of adrenocorticotrophin or dexamethasone to intact or hypophysectomized fetuses. *Journal of Endocrinology*, **75**, 59–71.

Kendall, J.Z., Richards, G.E. and Frankowski, R.F. (1983) Regulation of prolactin secretion in the pregnant pig: effect of fetal hypophysectomy and fetal death in small litters. *Biology of Reproduction*, **29**, 412–421.

Kendall, J.Z., Dziuk, P.J., Nelson, D.R. and Thurmon, J.C. (1985) The effect of fetal hypophysectomy in gilts with small litters on plasma estradiol 17β concentrations (abstract). *Biology of Reproduction*, **32**, Suppl. 1. 158.

Kennedy, P.C., Kendrick, J.W. and Stormont, C. (1957) Adenohypophysial aplasia, an inherited defect associated with abnormal gestation in Guernsey cattle. *Cornell Veterinarian*, **47**, 160–178.

Kennedy, P.C., Liggins, G.C. and Holm, L.W. (1967) Prolonged gestation, in, *Comparative Aspects of Reproductive Failure*, (ed. K. Benirscke), Springer-Verlag, New York, pp. 186–193.

Kertiles, L.P. and Anderson, L.L. (1979) Effect of relaxin on cervical dilation, parturition and lactation in the pig. *Biology of Reproduction*, **21**, 57–68.

Kidder, H.E., Casida, L.E. and Grummer, R.H. (1955) Some effects of estrogen injections on the estrual cycle of gilts. *Journal of Animal Science*, **14**, 470–476.

Kiesel, G.K. and Autrey, K.M. (1961) Relationship of progesterone to prolonged gestation in cattle.

Journal of the American Veterinary Association, **138**, 557–558.

Kikukawa, T., Cowan, B.D., Tejada, R. and Mukherjee, A.B. (1988) Partial characterisation of a uteroglobin-like protein in the human uterus and its temporal relationship to prostaglandin levels in this organ. *Journal of Clinical Endocrinology and Metabolism*, **67**, 315–320.

Kirton, K.T., Phariss, B.B. and Forbes, A.D. (1970) Some effects of prostaglandin E_2 and $F_{2\alpha}$ on the pregnant rhesus monkey. *Biology of Reproduction*, **3**, 163–168.

Klein, M. (1967) Sur le maintien de l'activité fonctionelle du corps jaune chez la lapine. *Archive Anatomique et Microscopique*, **56**, 292–299.

Knifton, A. (1967) Hormonal control of the rat myometrium. *Journal of Pharmacy and Pharmacology*, **19**, 767–768.

Korda, A.R., Challis, J.J., Anderson, A.B.M. and Turnbull, A.C. (1975) Assessment of placental function in normal and pathological pregnancies by estimation of plasma oestradiol levels after injection of dehydroepiandrosterone sulphate. *British Journal of Obstetrics and Gynaecology*, **82**, 656–661.

Kovacs, L., Sas, M., Resch, B.A., Ugocsai, G., Swahn, M.L., Bygdeman, M. and Rowe, P.J. (1984) Termination of very early pregnancy by RU486 – an antiprogestational compound. *Contraception*, **29**, 399–410.

Kraeling, R.R. and Davis, B.J. (1974) Termination of pregnancy by hypophysectomy in the pig. *Journal of Reproduction and Fertility*, **36**, 215–217.

Kraeling, R.R., Barb, C.R. and Davis, B.J. (1975) Prostaglandin-induced regression of porcine corpora lutea maintained by estrogen. *Prostaglandins*, **9**, 459–462.

Kreitmann, B. and Bayard, F. (1979) Oestrogen and progesterone receptor concentration in human endometrium during gestation. *Acta Endocrinologica*, **92**, 547–552.

Krishnan, R.S. and Daniel, J.C. (1967) 'Blastokinin': inducer and regulator of blastocyst development in the rabbit uterus. *Science*, **158**, 490–496.

Krug, E.C., Rogol, A.D., Jarvis, W.D., Thiagarajah, S. and Singhas, C.A. (1983) Prolactin secretion by human chorion–decidua *in vitro*. *American Journal of Obstetrics and Gynecology*, **147**, 38–42.

Kumar, D., Goodno, J.A. and Barnes, A.C. (1962) Studies in human myometrium during pregnancy. *American Journal of Obstetrics and Gynecology*, **84**, 1111–1116.

Kumar, D., Sullivan, W.J. and Wagatsuma, T. (1967) The mechanism of action of progesterone on human myometrium. *American Journal of Obstetrics and Gynecology*, **98**, 1055–1060.

Kumaresan, P., Anandarangam, P.B., Dianzon, W. and Vasicka, A. (1974) Plasma oxytocin levels during human pregnancy and labor as determined by radioimmunoassay. *American Journal of Obstetrics and Gynecology*, **119**, 215–223.

Kuriyama, H. (1961) The effect of progesterone and oxytocin on the mouse myometrium. *Journal of Physiology (London)*, **159**, 26–39.

Laatikainen, T., Pelkonen, J., Apter, D. and Ranta, T. (1980) Fetal and maternal serum levels of steroid sulfates, unconjugated steroids, and prolactin at term pregnancy and in early spontaneous labor. *Journal of Clinical Endocrinology and Metabolism*, **50**, 489–494.

Labhsethwar, A.P. and Watson, D.J. (1975) Temporal relationship between secretory patterns of gonadotropins, estrogens, progestins and PGF in periparturient rats. *Biology of Reproduction*, **10**, 103–110.

Lacroix, M.C. and Martal, J. (1979) Mise en évidence et evolution d'une gonadotrophine chorionique chez la Brebis. *Adademie des Sciences, Paris, Comptes Rendus Serie D*, **288**, 771–774.

van Landeghem, A.A.J. and van de Wiel, D.F.M. (1978) Radioimmunoassay for porcine prolactin:plasma levels during lactation, suckling and weaning and after TRH administration. *Acta Endocrinologica (Copenhagen)*, **88**, 653–667.

Landgraf, R., Shulz, J., Eulenberger, K. and Wilhelm, J. (1983) Plasma levels of oxytocin and vasopressin before, during and after parturition in cows. *Experimental and Clinical Endocrinology*, **81**, 321–328.

Lanman, J.T., Thau, R., Sundaram, K., Brinson, A. and Bonk, R. (1975) Ovarian and placental origins of plasma progesterone following fetectomy in monkey (*Macaca mulatta*). *Endocrinology*, **96**, 591–597.

Larson, J.W., Janson, T.M., Caldwell, B.V. and Speroff, L. (1973) The effect of estradiol infusion on uterine activity and peripheral levels of prostaglandin F and progesterone. *American Journal of Obstetrics and Gynecology*, **117**, 276–279.

Lau, I.F., Saksena, S.K. and Salmonsen, R. (1982) The concentration of progesterone, 20α-dihydroprogesterone, testosterone, oestrone and oestradiol-17β in serum, amniotic fluid and

placental tissue of pregnant rabbits. *Acta Endocrinologica*, **99**, 605–611.

Laudanski, T., Batra, S. and Akerlund, M. (1979) Prostaglandin-induced luteolysis in pregnant and pseudopregnant rabbits and the resultant effects on the myometrial activity. *Journal of Reproduction and Fertility*, **56**, 141–148.

Leach-Harper, C.M. and Thorburn, G.D. (1984) Inhibition of prostaglandin synthesis by ovine allantoic fluid: acute reduction in inhibitory activity during late gestation. *Canadian Journal of Physiology and Pharmacology*, **62**, 1152–1157.

Leake, R.D., Weitzmann, R.E., Glatz, T.H. and Fisher, D.A. (1981) Plasma oxytocin concentrations in men, non-pregnant women, and pregnant women before and during spontaneous labor. *Journal of Clinical Endocrinology and Metabolism*, **53**, 730–733.

Leaver, H.A. and Richmond, D.H. (1984) The effect of oxytocin, estrogen, calcium ionophore A23187 and hydrocortisone on prostaglandin $F_{2\alpha}$ and 6-oxo-prostaglandin $F_{1\alpha}$ production by cultured human endometrial and myometrial explants. *Prostaglandins, Leukotrienes and Medicine*, **13**, 179–196.

Ledger, W.L., Webster, M.A., Anderson, A.B. and Turnbull, A.C. (1985) Effect of inhibition of prostaglandin synthesis on cervical softening and uterine activity during ovine parturition resulting from progesterone withdrawal induced by epostane. *Journal of Endocrinology*, **105**, 227–233.

Legrand, C., Maltier, J.P. and Benghan-Eyene, Y. (1987) Rat myometrial adrenergic receptors in late pregnancy. *Biology of Reproduction*, **37**, 641–650.

Levitt, T.M., Tobon, H. and Josimovich, J.B. (1975) Prostaglandin content of human endometrium. *Fertility and Sterility*, **25**, 211–216.

Liggins, G.C. (1973) Hormonal interactions in the mechanism of parturition, in, *Memoirs of the Society for Endocrinology Endocrine: Factors in Labour* (eds A. Klopper and J. Gardner), Cambridge University Press, pp. 119–139.

Liggins, G.C. (1988) The onset of labour: an historical review, in *Fetal and Neonatal Development*, (ed. C.T. Jones), Perinatology Press, Ithaca, pp. 381–388.

Liggins, G.C. and Grieves, S. (1971) Possible role for prostaglandin $F_{2\alpha}$ in parturition in sheep. *Nature*, **232**, 629–631.

Liggins, G.C., Grieves, S.A., Kendall, J.Z. and Knox, B.S. (1972) The physiological roles of progesterone, oestradiol-17β and prostaglandin $F_{2\alpha}$ in the control of ovine parturition. *Journal of Reproduction and Fertility*, Suppl. 16, 85–103.

Liggins, G.C., Fairclough, R.J., Grieves, S.A., Kendall, J.Z. and Knox, B.S. (1973) The mechanism of initiation of parturition in the ewe. *Recent Progress in Hormone Research*, **29**, 111–159.

Liggins, G.C., Fairclough, R.J., Grieves, S.A., Froster, C.S. and Knox, B.S. (1977) Parturition in the sheep, in *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 5–30.

Liggins, G.C., Campos, G.A., Roberts, C.M. and Skinner, S.J. (1980) Production rates of prostaglandin F, 6-keto-$PGF_{1\alpha}$ and thromboxone B_2 by perifused human endometrium. *Prostaglandins*, **19**, 461–477.

Lincoln, D.W. (1971) Labour in the rabbit: Effect of electrical stimulation applied to the infundibulum and median eminence. *Journal of Endocrinology*, **84**, 244–251.

Linzell, J.L. and Heap, R.B. (1968) A comparison of progesterone metabolism in the pregnant goat and sheep: sources of production and a comparison of uptake by some target organs. *Journal of Endocrinology*, **41**, 433–438.

Lohse, J.K. and First, N.L. (1981) Development of the porcine fetal adrenal in late gestation. *Biology of Reproduction*, **25**, 181–190.

Long, G., Mansfield, S., Bicknell, R.J., Dean, A.D.P., Ingram, C.D., Marsh, M.I.C., Yates, J.O. and Dyer, R.G. (1985) Central opioids, a possible role in parturition. *Journal of Endocrinology*, **106**, 219–224.

Longcope, C., Layne, D.S. and Tait, J.F. (1968) Metabolic clearance rates and interconversions of estrone and 17-beta-estradiol in normal males and females. *Journal of Clinical Investigation*, **47**, 93–106.

Longenecker, D.E. and Day, B.N. (1972) Maintenance of corpora lutea and pregnancy in unilaterally pregnant pigs by intrauterine infusion of embryonic tissue. *Journal of Reproduction and Fertility*, **31**, 171–177.

Lopez-Bernal, A., Bryant-Greenwood, G.D., Hansell, D.J., Hicks, B.R., Greenwood, F.C. and Turnbull, A.C. (1987a) Effect of relaxin on prostaglandin E production by human amnion, changes in relation to the onset of labour. *British Journal of Obstetrics and Gynaecology*, **94**, 1045–1051.

Lopez-Bernal, A., Hansel, D.J., Alexander, S. and

Turnbull, A.C. (1987b) Steroid conversion and prostaglandin production by chorionic and decidual cells in relation to term and preterm labour. *British Journal of Obstetrics and Gynaecology*, **94**, 1052–1058.

Lopez-Bernal, A., Newman, G.E., Phizackerley, P.J.R. and Turnbull, A.C. (1988) Surfactant stimulates prostaglandin E production by human amnion. *British Journal of Obstetrics and Gynaecology*, **95**, 1013–1017.

Lopez-Bernal, A., Canete-Soler, R. and Turnbull, A.C. (1989) Are leukotrienes involved in human uterine contractility? *British Journal of Obstetrics and Gynaecology*, **96**, 568–573

Lye, S.J. and Porter, D.G. (1978) Demonstration that progesterone 'blocks' uterine activity in the ewe *in vivo* by a direct action on the myometrium. *Journal of Reproduction and Fertility*, **52**, 87–94.

Lytton, F.D.C. and Poyser, N.L. (1982) Concentrations of $PGF_{2\alpha}$ and PGE_2 in the uterine venous blood of rabbits during pseudopregnancy and pregnancy. *Journal of Reproduction and Fertility*, **64**, 421–429.

McCoshen, J.A. (1988) Associations between prolactin, prostaglandin E_2 and fetal membrane function in human gestation, in *The Physiology and Biochemistry of Human Fetal Membranes*, (ed. B.F. Mitchell), Perinatology Press, Ithaca, pp. 117–139.

McCoshen, J.A., Johnson, K.A., Dubin, N.H. and Ghodganonkar, P.B. (1987) Prostaglandin E_2 release on the fetal and maternal sides of the amnion and chorion–decidua before and after term labor. *American Journal of Obstetrics and Gynecology*, **56**, 173–178.

Macdonald, A.A., Elsaesser, F., Parvizi, N., Heilhecker, A., Smidt, D. and Ellendorff, F. (1979) Progesterone, oestradiol-17β and luteinizing hormone concentrations in the blood and amniotic fluid of chronically catheterized pig foetuses. *Journal of Endocrinology*, **80**, 14P.

Macdonald, A.A., Colenbrander, B., Elsaesser, F. and Heilhecker, A. (1980) Progesterone production by the pig fetus and the response to stimulation by adrenocorticotrophin. *Journal of Endocrinology*, **85**, 34P.

McDonald, L.E. and Hays, R.L. (1958) The effects of prepartum administration of progesterone to the cow. *American Journal of Veterinary Research*, **19**, 97–98.

McDonald, L.E., McNutt, S.H. and Nicholas, R.E. (1953) On the essentiality of the bovine corpus luteum of pregnancy. *American Journal of Veterinary Research*, **14**, 539–541.

Mackenzie, L.W. and Garfield, R.E. (1986) Effects of 17 beta-estradiol on myometrial gap junctions and pregnancy in the rat. *Canadian Journal of Physiology and Pharmacology*, **64**, 462–466.

MacLennan, A.H., Green, R.C., Bryant-Greenwood, G.D., Greenwood, F.C. and Seamark, R.F. (1980) Ripening of the human cervix and induction of labour with purified porcine relaxin. *Lancet*, **1**, 220–223.

MacLennan, A.H., Grant, P., Ness, D. and Down, A. (1986) Effect of porcine relaxin and progesterone on rat, pig and human myometrial activity *in vitro*. *Journal of Reproductive Medicine*, **31**, 43–49.

Madill, D. and Bassett, J.M. (1973) Corticosteroid release by adrenal tissue from foetal and newborn lambs in response to corticotrophin stimulation in a perifusion system *in vitro*. *Journal of Endocrinology*, **58**, 75–87.

Magyar, D.M., Fridshal, D., Elsner, C.W., Glatz, T., Eliot, J., Kelin, A.H., Lowe, K.C., Buster, J.E. and Nathanielsz, P.W. (1980) Time-trend analysis of plasma cortisol concentrations in the fetal sheep in relation to parturition. *Endocrinology*, **107**, 155–159.

Magyar, D.M., Elsner, C.W., Fridshal, D., Eliot, J., Klein, A., Glatz, T. Lowe, K.C., Nathanielsz, P.W. and Buster. J.E. (1981) Time trend analysis of plasma 11-desoxycorticosterone, corticosterone, cortisol and aldosterone in fetal and maternal sheep during the last 18 days of gestation. *Journal of Steroid Biochemistry*, **14**, 1091–1099.

Makarainen, L. and Ylikorkala, O. (1984) Amniotic fluid 6-keto- prostaglandin $F_{1\alpha}$ and thromboxane B_2 during labor. *American Journal of Obstetrics and Gynecology*, **150**, 765–768.

Makimura, N., Kako, K., Seki, K. and Mitsui, C. (1984) Changes in the concentrations of plasma steroid hormones and plasma 13,14-dihydro-15-oxo-prostaglandin $F_{2\alpha}$ in late pregnancy rabbits treated with an inhibitor of 3β-hydroxysteroid dehydrogenase. *Endocrinologica Japonica*, **31**, 349–353.

Makowski, E.L., Meschia, G., Droegemueller, W. and Battaglia, F.C. (1968) Distribution of uterine blood flow in the pregnant sheep. *American Journal of Obstetrics and Gynecology*, **101**, 409–412.

Malecki, J.C. (1989) The role of luteotrophins in the maintenance of pregnancy in the goat. *PhD Thesis*, Monash University, Melbourne, Australia.

Malecki, J., Jenkin, G. and Thorburn, G.D. (1987) Passive immunization of pregnant goats against ovine LH. *Journal of Endocrinology*, **114**, 413–436.

Mann, M.R., Curet, L.B. and Colas, A.E. (1975) Aromatizing activity of placental microsomal fractions from ewes in late gestation. *Journal of Endocrinology*, **65**, 117–125.

Manzai, M. and Liggins, G.C. (1984) Inhibitory effects of dispersed amnion cells on production rates of prostaglandin E and F by endometrial cells. *Prostaglandins*, **28**, 297–304.

Marsh, J.M. (1971) The effect of prostaglandins on the adenyl cyclase of the bovine corpus luteum. *Annals of the New York Academy of Sciences*, **180**, 416–425.

Marshall, P.J., Kilmacz, R.J. and Lands, W.E.M. (1987) Constraints on prostaglandin biosynthesis in tissues. *Journal of Biological Chemistry*, **262**, 3510–3517.

Martin, P.A., Norton, H.W. and Dziuk, P.J. (1977) The effect of corpora lutea induced during pregnancy on the length of gestation in the pig. *Biology of Reproduction*, **17**, 712–717.

Martin, P.A., Bevier, G.W. and Dziuk, P.J. (1978) The effect of disconnecting the uterus and ovary on the length of gestation in the pig. *Biology of Reproduction*, **18**, 428–433.

Mason, J.I., France, J.T., Magness, R.R., Murry, B.A. and Rosenfeld, C.R. (1989) Ovine placental steroid 17α-hydroxylase/C-17, 20-lyase, aromatase and sulphatase in dexamethasone-induced and natural parturition. *Journal of Endocrinology*, **122**, 351–359.

Mathius, J.B. and Kelly, R.W. (1978) Concentration of prostaglandins $F_{2\alpha}$ and E_2 in endometrium throughout the menstrual cycle, after the administration of clomiphene or an oestrogen-progestagen pill and in early pregnancy. *Journal of Endocrinology*, **77**,
361–371.

Matt, D.W. and Macdonald, G.J. (1984) *In vitro* progesterone and testosterone production by rat placenta during pregnancy. *Endocrinology*, **115**, 741–747.

Maule Walker, F.M. and Peaker, M. (1978) Production of oestradiol-17β by the goat mammary gland during late pregnancy in relation to lactation. *Journal of Physiology, (London)*, **284**, 71P–72P.

Meinecke-Tillman, S., Gips, H., Meinecke, B. and Finkenberg, A. (1986) Concentrations of oestrone and oestradiol-17α in the peripheral plasma of the nanny goat during the oestrous cycle, gestation and puerperium. *Zuchthygiene*, **21**, 207–213.

Meites, J., Webster, H.D., Young, F.W., Thorp, F. and Hatch, R.N. (1951) Effects of corpora lutea removal and replacement with progesterone on pregnancy in goats. *Journal of Animal Science*, **10**, 411–416.

Melin, P., Trojnar, J., Johansson, B., Vilhardt. H. and Akerlund, M. (1986) Synthetic antagonists of the myometrial response to vasopressin and oxytocin. *Journal of Endocrinology*, **111**, 125–131.

du Mesnil du Buisson, F. (1966) Contribution a l'étude due maintien du corps jaune de la truie. *These*, Paris.

du Mesnil du Buisson, F. (1973) Facteurs luteotropes chez la truie, in *Le Corps Jaune*, (eds R. Denamur and A. Netter), Masson, Paris, pp. 225–237.

du Mesnil du Buisson, F. and Dauzier, I. (1957) Influence de l'ovariectomie chez la Truie pendant la gestation. *Compte Rendus des Séances de la Société de Biologie*, **151**, 311–313.

du Mesnil du Buisson, F. and Rombauts, P. (1963) Réduction expérimentale du nombre des foetus au cours de la gestation. *Annales de Biologia Animale, Biochimie et Biophysique*, **3**, 445.

du Mesnil du Buisson, F. and Denamur, R. (1969) Mechanisme due contrôle de la function luteale chez la truie, la brebis et la vache, in, *Progress in Endocrinology*, (ed. C. Gual C), Excerpta Medica, Amsterdam, pp. 927–934.

Mitchell, B.F., Lye, S.J., Lukash, L. and Challis, J.R. (1986) Androstenedione metabolism in the late gestation sheep fetus. *Endocrinology*, **118**, 63–68.

Mitchell, B.F., Challis, J.R.G. and Lukash, L. (1987) Progesterone synthesis by human amnion, chorion, and decidua at term. *American Journal of Obstetrics and Gynecology*, **157**, 349–353.

Mitchell, M.D. (1987) Epidermal growth factor actions on arachidonic acid metabolism in human amnion cells. *Biochemica et Biophysica Acta*, **928**, 204–242.

Mitchell, M.D. and Flint, A.P. (1977) Prostaglandin concentrations in intra-uterine tissues from late pregnant sheep before and after labour. *Prostaglandins*, **14**, 563–569.

Mitchell, M.D. and Flint, A.P.F. (1978a) Use of meclofenamic acid to investigate the role of prostaglandin biosynthesis during induced parturition in sheep. *Journal of Endocrinology*, **76**, 101.

Mitchell, M.D. and Flint, A.P.F. (1978b)

Prostaglandin production by intra-uterine tissues from periparturient sheep, use of a superfusion technique. *Journal of Endocrinology*, **76**, 111–121.

Mitchell, M.D., Flint, A.P.F. and Turnbull, A.C. (1975) Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep. *Prostaglandins*, **9**, 47–56.

Mitchell, M.D., Patrick, J.E., Robinson, J.S., Thorburn, G.D. and Challis, J.R.G. (1976) Prostaglandins in the plasma and amniotic fluid of rhesus monkeys during pregnancy and after intra-uterine foetal death. *Journal of Endocrinology*, **71**, 67–76.

Mitchell, M.D., Robinson, J.S. and Thorburn, G.D. (1977a) Prostaglandins in fetal tracheal and amniotic fluid from late pregnant sheep. *Prostaglandins*, **14**, 1006–1011.

Mitchell, M.D., Flint, A.P.F. and Turnbull, A.C. (1977b) Stimulation of uterine activity by administration of prostaglandin $F_{2\alpha}$ during parturition in sheep. *Journal of Reproduction and Fertility*, **48**, 189–190.

Mitchell, M.D., Keirse, M.J.N.C., Anderson, A.B.M. and Turnbull, A.C. (1978a) Thromboxane B_2 in amniotic fluid before and during labour. *British Journal of Obstetrics and Gynaecology*, **85**, 442–445.

Mitchell, M.D., Flint, A.P., Bibby, J., Brunt, J., Arnold, J.M., Anderson, A.B.M. and Turnbull, A.C. (1978b) Plasma concentrations of prostaglandins during late human pregnancy, Influence of normal and preterm labour. *Journal of Clinical Endocrinology and Metabolism*, **46**, 947–951.

Mitchell, M.D., Bibby, J., Hicks, B.R. and Turnbull, A.C. (1978c) Specific production of prostaglandin E by human amnion *in vitro*. *Prostaglandins*, **15**, 377–382.

Mitchell, M.D., Clover, L., Thorburn, G.D. and Robinson, J.S. (1978d) Specific change in the direction of prostaglandin synthesis by intrauterine tissues of the rhesus monkey (*Macaca mulatta*) during late pregnancy. *Journal of Endocrinology*, **78**, 343–350.

Mitchell, M.D., Ellwood, D.A., Anderson, A.B.M. and Turnbull, A.C. (1978e) Elevated concentrations of 6-keto-prostaglandin F_1 alpha in fetal and maternal plasma and in amniotic fluid during ovine parturition. *Prostaglandins and Medicine*, **1**, 265–266.

Mitchell, M.D., Flint, A.P.F., Robinson, J.S. and Thorburn, G.D. (1978f) *In vitro* production of prostaglandins by intrauterine tissues from pregnant goats. *Journal of Reproduction and Fertility*, **52**, 151–152.

Mitchell, M.D., Keirse, M.J.N.C., Anderson, A.B.M. and Turnbull, A.C. (1979a) Concentrations of the prostacyclin metabolite, 6-keto-prostaglandin $F_{1\alpha}$ in amniotic fluid during late pregnancy and labour. *British Journal of Obstetrics and Gynaecology*, **86**, 350–353.

Mitchell, M.D., Anderson, A.B., Brunt, J.D., Clover, L., Ellwood, D.A., Robinson, J.S. and Turnbull, A.C. (1979b) Concentrations of 6-oxo-prostaglandin F_1 alpha in the maternal and foetal plasma of sheep during spontaneous and induced parturition. *Journal of Endocrinology*, **83**, 141–148.

Mitchell, M.D., Jamieson, D.R.S., Sellers, S.M. and Turnbull, A.C. (1980a) 6-keto-$PGF_{1\alpha}$: Concentrations in human umbilical plasma and production by umbilical vessels, in, *Advances in Prostaglandin and Thromboxane Research*, (eds B. Samuelsson, P.W. Ramwell and R. Paoletti), Vol. 7, Raven Press, New York, pp. 891–895.

Mitchell, M.D., Mountford, L.A., Natale, R. and Robinson, J.S. (1980b) Concentrations of oxytocin in the plasma and amniotic fluid of rhesus monkeys (*Macaca mulatta*) during the latter half of pregnancy. *Journal of Endocrinology*, **84**, 473–478.

Mitchell, M.D., Brunt, J., Clover, L. and Walker, D.W. (1980c) Prostaglandins in the umbilical and uterine circulations during late pregnancy in the ewe. *Journal of Reproduction and Fertility*, **58**, 293–297.

Mitchell, M.D., Ebenhack, K., Kraemer, D.L., Cox, K., Cutner, S. and Strickland, D.M. (1982a) A sensitive radioimmunoassay for 11-deoxy-13,14 - dihydro - 15 - keto - 11,16 - cyclo - prosta - glandin E_2, application as an index of prostaglandin E_2 biosynthesis during human pregnancy and parturition. *Prostaglandins, Leukotrienes and Medicine*, **9**, 949–557.

Mitchell, M.D., Webb, R., Brennecke, S.P., Anderson, A.B. and Turnbull, A.C. (1982b). Oxytocin in the fetal and maternal circulations during induced and spontaneous parturition in sheep. *European Journal of Obstetric and Gynecological Reproductive Biology*, **14**, 115–120.

Mitchell, M.D., Brennecke, S.P. and Novy, M.J. (1984) Prostaglandin synthase inhibitory activity in the plasma of rhesus monkeys during late pregnancy: Effect of dexamethasone. *Prostaglandins, Leukotrienes and Medicine*, **14**, 199–204.

Mitchell, M.D., Edwin, S. and Romero, R.J. (1990) Prostaglandin biosynthesis by human decidual cells, Effects of inflammatory mediators. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **41**, 35–38.

Morishita, S. (1986) Prompt effect of progesterone on the adrenergic response of smooth muscles. *Japanese Journal of Pharmacology*, **42**, 289–296.

Mortimer, G., Stimson, W.H., Hunter, I.C. and Govan, A.D.T. (1985) A role for amniotic epithelium in control of human parturition. *Lancet*, **1**, 1074–1075.

Mueh, J., Shane, J.M. and Naftolin, F. (1975) The effects of methallibure (ICI 33828) on the LH release in castrate male rats challenged with LHRH. *Endocrinology*, **97**, 493–495.

Mulay, S., Giannopoulos, G. and Solomon, S. (1973) Corticosteroid levels in the mother and fetus of the rabbit during gestation. *Endocrinology*, **93**, 1342–1348.

Musah, A.I., Schwabe, C., Willham, R.L. and Anderson, L.L. (1986) Relaxin on induction of parturition in brief heifers. *Endocrinology*, **118**, 1476–1482.

Musah, A.I., Schwabe, C. and Anderson, L.L. (1989) Relaxin regulates oxytocin secretion in late-pregnant beef heifers. *Proceedings of the Society for Experimental Biology and Medicine*, **191**, 124–129.

Myatt, L., Rose, M.P. and Elder, M.G. (1985) Lipoxygenase products of human fetal membranes. *32nd Annual Meeting of the Society for Gynecological Investigation*, abstract 160.

Nagata, J., Sunaga, H., Furuya, K., Makimura, N. and Kato, K. (1987) Changes in the plasma prostaglandin $F_{2\alpha}$ metabolite before and during spontaneous labor and labor induced by amniotomy, oxytocin and prostaglandin E_2. *Endocrinologica Japonica*, **34**, 153–159.

Nakano, R., Akahori, T., Katayama, K. and Tojo, S. (1977) Binding of LH and FSH to porcine granulosa cells during follicular maturation. *Journal of Reproduction and Fertility*, **51**, 23–27.

Nakla, S., Skinner, K., Mitchell, B.F. and Challis, J.R.G. (1986) Changes in prostaglandin transfer across human fetal membranes obtained after spontaneous labor. *American Journal of Obstetrics and Gynecology*, **155**, 1337–1341.

Nancarrow, C.D. (1969) Gestagen metabolism in the ovine foetal-placental unit. *PhD Thesis*, University of Adelaide, South Australia.

Nancarrow, C.D. and Seamark, R.F. (1968) Progesterone metabolism in fetal blood. *Steroids*, **12**, 367–379.

Nara, B.S. and First, N.L. (1977) Effect of indomethacin and PGF_2 on porcine parturition. *Journal of Animal Science*, **45**, 191.

Nara, B.S. and First, N.L. (1981) Effect of indomethacin and prostaglandin F_2 alpha on parturition in swine. *Journal of Animal Science*, **52**, 1360–1370.

Nara, B.S., Darmadja, D. and First, N.L. (1981) Effect of removal of follicles, corpora lutea or ovaries on maintenance of pregnancy in swine. *Journal of Animal Science*, **52**, 794–801.

Nara, B.S., Ball, G.D., Rutherford, J.E., Sherwood, O.D. and First, N.L. (1982) Release of relaxin by a non-luteolytic dose of prostaglandin $F_{2\alpha}$ in pregnant swine. *Biology of Reproduction*, **27**, 1190–1195.

Nase, B.R., Hagen, D.R., Kavanaugh, J.F. and Griel, L.C. (1985) Effect of partial stepwise luteectomy in pregnant gilts on maternal and fetal concentrations of progesterone. *Biology of Reproduction*, **33**, 88–92.

Nathanielsz, P.W. and Abel, M. (1972) Cortisol-induced parturition in the rabbit. *Journal of Endocrinology*, **54**, 345–346.

Nathanielsz, P.W. and Abel, M. (1973) Initiation of parturition in the rabbit by maternal and foetal administration of cortisol: Effect of rate and duration of administration: suppression of delivery by progesterone. *Journal of Endocrinology*, **57**, 47–54.

Nathanielsz, P.W., Abel, M. and Smith, G.W. (1973) Hormonal factors in parturition in the rabbit, in, *Fetal and Neonatal Physiology*, (eds R.S. Comline, K.W. Cross, G.S. Dawes and P.W. Nathanielsz), Cambridge University Press, Cambridge, pp. 594–602.

Nathanielsz, P.W., Jack, P.M.B., Krane, E., Thomas, A.L., Ratter, S. and Rees, L.H. (1977) The role and regulation of corticotropin in the fetal sheep, in, *The Fetus and Birth*, (eds J. Knight and M. O'Connor) Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 73–98.

Nathanielsz, P.W., Elsner, C., Magyar, D., Fridshal, D., Freeman, A. and Buster, J.E. (1982) Time trend analysis of plasma unconjugated and sulfoconjugated estrone and 3B-δ5-steroids in fetal and maternal sheep plasma in relation to spontaneous parturition at term. *Endocrinology*, **110**, 1402–1407.

Nathanielsz, P.W., Buster, J.E., Jenkin, G., Jorgensen, G. and Thorburn, G.D. (1988)

Induction of premature delivery in sheep following infusion of cortisol to the fetus: the effect of maternal progestagen treatment on the C21-steroid-17α-hydroxylase, C-17,20-lyase and aromatase pathways. *Journal of Developmental Physiology*, **10**, 257–270.

Nathanielsz, P.W., Honnebier, M.B.O.M. and Figueroa, J.P. (1990) Prolongation of gestation in pregnant rhesus monkeys following fetectomy. *Journal of Physiology*, **430**, 79P.

Nellor, J.E. (1963) Induced delayed parturition in swine and cattle. *Physiologist*, **6**, 244.

Niesert, S., Christopherson, W., Korte, K., Mitchell, M.D., MacDonald, P.C. and Casey, M.L. (1986) Prostaglandin E_2 9-ketoreductase activity in human decidua vera tissue. *American Journal of Obstetrics and Gynecology*, **155**, 1348–1352.

Nishihira, J., Ishibashi, T., Imai, Y., Muramatsu, T. (1984) Mass spectrometric evidence for the presence of platelet-activating factor (1-*O*-alkyl-2-acetyl-sn-glycerophosphocholine) in human amniotic fluid. *Lipids*, **19**, 907–910.

Nissenson, R., Fluoret, G. and Hechter, D. (1978) Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus. *Proceedings of the National Academy of Sciences of the USA*, **75**, 2044–2048.

North, S.A., Hauser, E.R. and First, N.L. (1973) The induction of parturition in swine and rabbits with corticosteroid dexamethasone. *Journal of Animal Science*, **36**, 1170–1174.

Novy, M.J. and Walsh, S.W. (1983) Dexamethasone and estradiol treatment in pregnant rhesus macaques: Effects on gestational length, maternal plasma hormones, and fetal growth. *American Journal of Obstetrics and Gynecology*, **145**, 920–926.

Novy, M.J. and Haluska, G.J. (1988) Endocrine and paracrine control of parturition in rhesus monkeys, in *The Onset of Labor: Cellular and Integrative Mechanisms*, (eds D. McNellis, J. Challis, P. MacDonald, P. Nathanielsz and J. Roberts), Perinatology Press, Ithaca, pp. 321–334.

Novy, M.J., Cook, M.J. and Manaugh, L.C. (1974) Indomethacin block of normal onset of parturition in primates. *American Journal of Obstetrics and Gynecology*, **118**, 412–416.

Novy, M.J., Walsh, S.W. and Kittinger, G.W. (1977) Experimental fetal anencephaly in the rhesus monkey: effect on gestational length and fetal and maternal plasma steroids. *Journal of Clinical Endocrinology and Metabolism*, **45**, 1031–1038.

Nuti, K.M. and Karavolas, H.J. (1977) Effect of progesterone and its 5α-reduced metabolites on gonadotropin levels in estrogen-primed ovariectomized rats. *Endocrinology*, **100**, 777–781.

O'Bryne, K.T., Ring, J.P. and Summerlee, A.J. (1986) Plasma oxytocin and oxytocin neurone activity during delivery in rabbits. *Journal of Physiology*, **370**, 501–513.

Ohashi, M., Simpson, E.R., Kramer, R.E. and Carr, B.R. (1982) Regulation of low-density lipoprotein receptors in cultured bovine adrenocortical cells. *Archives of Biochemistry and Biophysics*, **215**, 199–205.

Okazaki, T., Okita, J.R., MacDonald, P.L. and Johnston, J.M. (1978) Initiation of human parturition. X. Substrate specificity of phospholipase A_2 in human fetal membranes. *American Journal of Obstetrics and Gynecology*, **130**, 432–438.

Okazaki, T., Casey, M.L., Bleasdale, J.E., Okita, J.R., MacDonald, P.C. and Johnston, J.M. (1981a) Initiation of human parturition. XIII. Phospholipase C, phospholipase A_2 , and diacylglycerol lipase activities in fetal membranes and decidua vera tissues from early and late gestation. *Journal of Biological Chemistry*, **256**, 7316–7321.

Okazaki, T., Casey, M.L., Okita, J.R., MacDonald, P.C. and Johnston, J.M. (1981b) Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. *American Journal of Obstetrics and Gynecology*, **139**, 373–381.

Okazaki, T., Sagawa, N., Bleadsdale, J.E., Okita, J.R., MacDonald, P.C. and Johnston, M.J. (1981c) Initiation of human parturition. XIII. Phospholipase C, phospholipase A_2 and diacylglycerol lipase activities in fetal membranes and decidua vera tissues from early and late gestation. *Biology of Reproduction*, **25**, 103–109.

Okazaki, T., Sagawa, N., Okita, J.R., Bleasdale, J.E., MacDonald, P.C. and Johnston, J.M. (1981d) Diacylglycerol metabolism and arachidonic acid release in human fetal membranes and decidua vera. *Journal of Biological Chemistry*, **256**, 7316–7321.

Okita, J.R., MacDonald, P.C. and Johnston, J.M. (1982a) Mobilization of arachidonic acid from specific glycerophospholipids of human fetal membranes during early labor. *Journal of Biological Chemistry*, **257**, 14029–14034.

Okita, J.R., MacDonald, P.C. and Johnson, J.M.

(1982b) Initiation of human parturition: XIV. Increase in the diacylglycerol content of amnion during parturition. *American Journal of Obstetrics and Gynecology*, **142**, 432–435.

Okita, J.R., Johnston, J.M. and MacDonald, P.C. (1983) Source of prostaglandin precursor in human fetal membranes: Arachidonic acid content of amnion and chorion laeve of diamniotic–dichorionic twin placentae. *American Journal of Obstetrics and Gynecology*, **147**, 477–482.

Olson, D.M., Skinner, K. and Challis, J.R.G. (1983) Prostaglandin output in relation to parturition by cells dispersed from human intrauterine tissues. *Journal of Clinical Endocrinology and Metabolism*, **57**, 694–699.

Olson, D.M., Lye, S.J., Skinner, K. and Challis, J.R. (1985) Prostanoid concentrations in maternal/fetal plasma and amniotic fluid and intrauterine tissue prostanoid output in relation to myometrial contractility during the onset of adrenocorticotropin-induced preterm labour in sheep. *Endocrinology*, **116**, 389–397.

Omini, C., Falco, G.C., Pasargiklian, R., Fano, M. and Berti, F. (1979) Prostacyclin (PGI_2) and its metabolite 6-oxo-$PGF_{1\alpha}$ in pregnant human uterus. *Prostaglandins*, **17**, 113–120.

Osa, T. and Ogasawara, T. (1984) Effects *in vitro* of progesterone and estradiol-17 beta on the contractile and electrical responses in rat myometrium. *Japanese Journal of Physiology*, **34**, 427–441.

Ottobre, J.S., Nixon, W.E. and Stouffer, R.L. (1984) Induction of relaxin secretion in rhesus monkeys by human chorionic gonadotropin: Dependence on the age of the corpus luteum of the menstrual cycle. *Biology of Reproduction*, **31**, 1000–1006.

Pakravan, P., Kenny, F.M., Depp, R. and Allen, A.C. (1974) Familial congenital absence of adrenal glands: evaluation of glucocorticoid, mineralocorticoid and estrogen production in the perinatal period. *Journal of Pediatrics*, **84**, 74–78.

Pardo, R.J. and Larkin, L.H. (1982) Localization of relaxin in endometrial gland cells of pregnant, lactating, and ovariectomized hormone-treated guinea pigs. *American Journal of Anatomy*, **164**, 79–90.

Pardo, R.J., Larkin, L.H. and Fields, P.A. (1980) Immunocytochemical localization of relaxin in endometrial glands of the pregnant guinea pig. *Endocrinology*, **107**, 2110–2112.

Parker, C.R., Leveno, K., Carr, B.R., Hauth, J. and MacDonald, P.C. (1982) Umbilical cord plasma levels of dihydroepiandrosterone sulfate during human gestation. *Journal of Clinical Endocrinology and Metabolism*, **54**, 1216–1220.

Parker, C.R., Illingworth, D.R., Bissonnette, J. and Carr, B.R. (1986) Endocrine changes during pregnancy in a patient with homozygous familial hypobetalipoproteinemia. *New England Journal of Medicine*, **314**, 557–560.

Pashen, R.L. and Allen, W.R. (1979) The role of the fetal gonads and placenta in steroid production, maintenance of pregnancy and parturition in the mare. *Journal of Reproduction and Fertility*, Suppl. 27, 499–504.

Pashen, R.L., Sheldrick, E.L., Allen, W.R. and Flint, A.P.F. (1982) Dehydroepiandrosterone synthesis in the fetal foal and its importance as an oestrogen precursor. *Journal of Reproduction and Fertility*, Suppl. 32, 389–397.

Pattison, N.S., Webster, M.A., Phipps, S.L., Anderson, A.B.M. and Gilmer, M.D.G. (1984) Inhibition of 3β-hydroxysteroid dehydrogenase (HSD) activity in first- and second-trimester human pregnancy and the luteal phase using epostane. *Fertility and Sterility*, **42**, 875–881.

Pavlik, E.J. and Coulson, P.B. (1976) Modulation of oestrogen receptors in four different target tissues: differential effects of oestrogen vs progesterone. *Journal of Steroid Biochemistry*, **7**, 369–376.

Perezgrovas, R. and Anderson, L.L. (1982) Effect of porcine relaxin on cervical dilatation, pelvic area and parturition in beef heifers. *Biology of Reproduction*, **26**, 765–776.

Peterson, A.J., Hunter, J.T., Welch, R.A.S. and Fairclough, R.J. (1975) Oestrogens in bovine fetal and maternal plasma near term. *Journal of Reproduction and Fertility*, **43**, 179–181.

Pierrepoint, C.G., Anderson, A.B., Harvey, G., Turnbull, A.C. and Griffiths, K. (1971) The conversion *in vitro* of C_{19} -steroids to oestrogen sulphates by the sheep placenta. *Journal of Endocrinology*, **50**, 537–538.

Pimentel, C.A., Vijayakumar, R., Weston, P.G., Pimentel, S.M., Wagner, W.C. and Hixon, J.E. (1986) Effects of estrone infusion on secretion of F series prostaglandins on function of the corpus luteum during late gestation in cattle. *American Journal of Veterinary Research*, **47**, 1972–1977.

Pimentel, S.M., Pimentel, C.A., Weston, P.G., Hixon, J.E. and Wagner, W.C. (1986) Progesterone secretion by the bovine fetoplacental unit and responsiveness of corpora lutea to steroidogenic stimuli at two stages of ges-

tation. *American Journal of Veterinary Research*, **47**, 1967–1971.

Pinto, R.M., Leon, C., Mazzocco, N. and Scasserra, V. (1967) Action of estradiol-17β at term and at onset of labor. *American Journal of Obstetrics and Gynecology*, **98**, 540–546.

Polge, C., Rowson, L.E.A. and Chang, M.C. (1966) The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig. *Journal of Reproduction and Fertility*, **12**, 395–397.

Pope, G.S., Gupta, S.K. and Munro, I.B. (1969) Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. *Journal of Reproduction and Fertility*, **20**, 369–381.

Pope, N.S., Sargeant, G.F., Wiseman, B.S. and Kesler, D.J. (1987) Transitory changes of hormones in the plasma of parturient pony mares. *Journal of Reproduction and Fertility*, Suppl. 35, 629–634.

Porter, D.G. (1970) The failure of progesterone to affect myometrial activity in the guinea pig. *Journal of Endocrinology*, **46**, 425–434.

Porter, D.G. (1971) Quantitative changes in myometrial activity in the guinea-pig during pregnancy. *Journal of Reproduction and Fertility*, **27**, 219–226.

Porter, D.G. (1972) Myometrium of the guinea pig: The probable importance of relaxin. *Biology of Reproduction*, **7**, 458–464.

Porter, D.G. (1979) The myometrium and the relaxin enigma. *Animal Reproduction Science*, **2**, 77–96.

Porter, D.G. and Challis, J.R.G. (1974) Failure of high uterine concentrations of progesterone to inhibit myometrial activity *in vivo* in the postpartum rat. *Journal of Reproduction and Fertility*, 39,157-162.

Porter, D.G. and Schofield, B.M. (1966) Intrauterine pressure changes during pregnancy and parturition in rabbits. *Journal of Endocrinology*, **36**, 291–299.

Porter, D.G. and Watts, A.D. (1986) Relaxin and progesterone are myometrial inhibitors in the ovariectomized non-pregnant mini-pig. *Journal of Reproduction and Fertility*, **76**, 205–213.

Porter, D.G., Downing, S.J. and Bradshaw, J.M.C. (1979) Relaxin inhibits spontaneous and prostaglandin driven myometrial activity in anaesthetized rats. *Journal of Endocrinology*, **83**, 183–192.

Power, S.G.A. and Challis, J.R.G. (1987) Tissue-specific concentration changes of estrone and estradiol during spontaneous and ACTH-induced parturition in sheep. *Canadian Journal of Physiology and Pharmacology*, **65**, 130–135.

Poyser, N.L. (1984) Prostaglandin production by the early pregnant guinea-pig uterus in relation to implantation and luteal maintenance, and the effect of oestradiol. *Journal of Reproduction and Fertility*, **72**, 117–127.

Pulkkinen, M.O. and Enkola, K. (1972) The progesterone gradient of the human fetal membranes. *International Journal of Gynecology and Obstetrics*, **10**, 93–94.

Pupkin, M.J., Schomberg, D.W., Nagey, D.A. and Crenshaw, C. Jr. (1975) Effect of exogenous dehydroepiandrosterone upon the fetoplacental biosynthesis of estrogens and its effect upon uterine blood flow in the term pregnant ewe. *American Journal of Obstetrics and Gynecology*, **121**, 227–232.

Quartero, H.W.P. and Fry, C.H. (1989) Placental corticotrophin releasing factor may modulate human parturition. *Placenta*, **1**, 439–443.

Quirk, S.M. and Currie, W.B. (1984) Uterine steroid receptor changes associated with progesterone withdrawal during pregnancy and pseudopregnancy in rabbits. *Endocrinology*, **114**, 182–191.

Raeside, J.I., Liptrap, R.M., McDonell, W.M. and Milne, F.J. (1979) A precursor role of DHA in a feto-placental unit for oestrogen formation in the mare. *Journal of Reproduction and Fertility*, Suppl. 27, 493–497.

Randall, G.C.B. (1983) Changes in the concentrations of corticosteroids in the blood of fetal pigs and their dams during late gestation and labour. *Biology of Reproduction*, **27**, 1077–1084.

Randall, G.C.B. (1989) Effect of hypophysectomy on body and organ weights and subsequent development in the fetal pig. *Canadian Journal of Animal Science*, **69**, 655–661.

Randall, G.C.B. and Tsang, B.K. (1986) Influence of the fetal pituitary–adrenal axis on fetal and maternal progesterone and unconjugated oestrogen concentrations in the pig. *Journal of Reproduction and Fertility*, **78**, 65–72.

Randall, G.C.B., Kendall, J.Z., Tsang, B.K. and Taverne, M.A.M. (1984) Role of the fetal adrenal in the timing of parturition in the pig. *Proceedings of the XIth International Congress of Animal Reproduction and Artificial Insemination*, Illinois, **2**, 108.

Randall, G.C.B., Taverne, M.A.M., Challis, J.R.G., Kendall, J.Z. and Tsang, B.K. (1986) Inter-relationships between endocrine changes

in peripheral and uterine-venous blood and uterine activity at parturition in the pig. *Animal Reproduction Science*, **11**, 283–294.

Randall, G.C.B., Kendall, J.Z., Tsang, B.K. and Taverne MAM (1990) Endocrine changes following infusion of fetal pigs with corticotropin in litters of reduced numbers. *Animal Reproduction Science*, **23**, 109–122.

Rasmussen, A.B., Johannesen, P., Allen, J., Rehnstrom, J. and Fuchs, A.R. (1985) Plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ levels in women during induction of labor with I.V. infusion of prostaglandin $F_{2\alpha}$ in relation to uterine contractions. *Journal of Perinatal Medicine*, **13**, 15–21.

Rawlings, N.C. and Ward, W.R. (1976) Changes in steroid hormones in plasma and myometrium and uterine activity in ewes during late pregnancy and parturition. *Journal of Reproduction and Fertility*, **48**, 355–360.

Reddi, K. and Norman, R.J. (1985) Prostaglandin inhibitory compounds in the initiation of human labour. *Lancet*, **1**, 1508.

Rehnstrom, J., Ishikawa, M., Fuchs, F. and Fuchs, A.R. (1983) Stimulation of myometrial and decidual prostaglandin production by amniotic fluid from term, but not midtrimester pregnancies. *Prostaglandins*, **26**, 973–981.

Reimer, R.K., Goldfien, A.C. and Roberts, J.M. (1986) Rabbit uterine oxytocin receptors and *in vitro* contractile response: abrupt changes at term and the role of eicosanoids. *Endocrinology*, **119**, 699–709.

Reimers, T.J., Ullmann, M.B. and Hansel, W. (1985) Progesterone and prostanoid production by bovine binucleate trophoblastic cells. *Biology of Reproduction*, **33**, 1227–1236.

van Rensburg, S.J. (1971) Reproductive physiology and endocrinology of normal and habitually aborting angora goats. Onderstepoort, *Journal of Veterinary Research*, **38**, 1–62.

Resko, J.A., Ploem, J.G. and Stadelman, H.L. (1975) Estrogens in fetal and maternal plasma of the rhesus monkey. *Endocrinology*, **97**, 425–430.

Rice, G.E. and Smirnis, M. (1990) Identification of luteinizing hormone releasing hormone-like immunoactivity in ovine cotyledons. *Regulatory Peptides*, **27**, 51–59.

Rice, G.E., Wong, M.H., Ralph, M.M. and Thorburn, G.D. (1987) Ovine allantoic inhibition of prostaglandin synthesis in cotyledonary microsomes. *Journal of Endocrinology*, **114**, 295–300.

Rice, G.E., Wong, M.H. and Thorburn, G.D. (1988) Gestational changes in prostaglandin synthase activity of ovine cotyledonary microsomes. *Journal of Endocrinology*, **118**, 265–270.

Rice, G.E., Christensen, P., Dantzer, V. and Skadhauge, E. (1989) Gestational profile of prostaglandin E_2 synthesis by porcine placenta and fetal membranes. *Eicosanoids*, **2**, 235–240.

Rice, G.E., Wong, M.H., Hollingworth, S. and Thorburn, G.D. (1990) Prostaglandin G/H synthase activity in ovine cotyledons: a gestational profile. *Eicosanoids*, **3**, 231–236.

Ricketts, A.P. and Flint, A.P.F. (1980) Onset of synthesis of progesterone by ovine placenta. *Journal of Endocrinology*, **86**, 337–347.

Ricketts, A.P., Sheldrick, E.L., Lindsay, K.S. and Flint, A.P.F. (1980) Induction of labour in sheep after fetal hypophysectomy: An investigation of the possible involvement of a fetal pituitary secretion in the activation of placental enzymes by fetal cortisol. *Placenta*, **1**, 287–297.

Risbridger, G.P., Leach Harper, C.M., Wong, M.M. and Thorburn, G.D. (1985) Gestational changes in prostaglandin production by ovine fetal trophoblast cells. *Placenta*, **6**, 117–126

Roberts, J.S. (1975) Cyclical fluctuations in reflexive oxytocin release during the estrous cycle of the goat. *Biology of Reproduction*, **13**, 314–317.

Roberts, J.S. and Share, L. (1968) Oxytocin in plasma of pregnant, lactating and cycling ewes during vaginal stimulation. *Endocrinology*, **83**, 272–278.

Roberts, J.S. and Share, L. (1969) Effects of progesterone and estrogen on blood levels of oxytocin during vaginal distension. *Endocrinology*, **84**, 1076–1081.

Roberts, J.S., McCracken, J.A., Gavagan, J.E. and Soloff, M.S. (1976) Oxytocin-stimulated release of prostaglandin F_2 alpha from ovine endometrium *in vitro*: correlation with estrous cycle and oxytocin-receptor binding. *Endocrinology*, **99**, 1107–1114.

Robertson, H.A. (1972) Sequential changes in plasma progesterone in the cow during the estrus cycle, pregnancy, at parturition and postpartum. *Canadian Journal of Animal Science*, **52**, 645–650.

Robertson, H.A. and Smeaton, T.C. (1973) The concentration of unconjugated oestrone, oestradiol-17 alpha and oestradiol-17 beta in the maternal plasma of the pregnant ewe in relation to the initiation of parturition and lactation. *Journal of Reproduction and Fertility*, **35**, 461–468.

Robertson, H.A. and King, G.J. (1974) Plasma concentrations of progesterone, oestrone, oestradiol-17β and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. *Journal of Reproduction and Fertility*, **40**, 133–141.

Robinson, B.G., Emanuel, R.L., Frim, D.M. and Majzoub, J.A. (1988) Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proceedings of the National Academy of Sciences of the USA*, **85**, 5244–5248.

Robinson, J.S., Challis, J.R.G., Pooley, G. and Thorburn, G.D. (1977) Foetal and maternal cortisol and progesterone and maternal oestradiol in prolonged pregnancy after foetal hypophysectomy in sheep. *Journal of Endocrinology*, **72**, 241–242.

Robinson, J.S., Chapman, R.L.K., Challis, J.R.G., Mitchell, M.D. and Thorburn, G.D. (1978) Administration of extra-amniotic arachidonic acid and the suppression of uterine prostaglandin synthesis during pregnancy in the rhesus monkey. *Journal of Reproduction and Fertility*, **54**, 369–373.

Rolland, R., Gunsalus, G.L. and Hammond, J.M. (1976) Demonstration of specific binding of prolactin by porcine corpora lutea. *Endocrinology*, **98**, 1083–1091.

Romano, W., Lukash, L., Challis, J.R.G. and Mitchell, B.F. (1986) Substrate utilization for estrogen synthesis by human fetal membranes and decidua. *American Journal of Obstetrics and Gynecology*, **155**, 1170–1175.

Romero, R., Emamian, M., Wan, M., Grzyboski, C., Hobbins, J.C. and Mitchell, M.D. (1987a) Increased concentrations of arachidonic acid lipoxygenase metabolites in amniotic fluid during parturition. *Obstetrics and Gynecology*, **70**, 849–853.

Romero, R., Kadar, N., Hobbins, J.C. and Duff, G.W. (1987b) Infection and labor: The detection of endotoxin in amniotic fluid. *American Journal of Obstetrics and Gynecology*, **157**, 815–819.

Romero, R., Lafreniere, D., Hobbins, J.C. and Mitchell, M.D. (1987c) A product from human decidua inhibits prostaglandin production by human amnion. *Prostaglandins, Leukotrienes and Medicine*, **30**, 29–35.

Romero, R., Brody, D.T., Oyarzun, E., Mazor, M., Wu, Y.K., Hobbins, J.C. and Durum, S.K. (1989a) Infection and labor. III. Interleukin-1: A signal for the onset of parturition. *American Journal of Obstetrics and Gynecology*, **160**, 1117–1123.

Romero, R., Manogue, K.R., Mitchell, M.D., Wu, Y.K., Oyarzun, E., Hobbins, J.C. and Cerami, A. (1989b) Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. *American Journal of Obstetrics and Gynecology*, **161**, 336–341.

Romero, R., Wu, Y.K., Brody, D.T., Oyarzun, E., Duff, G.W. and Durum, S.K. (1989c) Human decidua: a source of interleukin-1. *Obstetrics and Gynecology*, **73**, 31–34.

Rosenfeld, C.R. and Worley, R.J. (1978) The effect of systemic infusions of dehydroisoandrosterone on the distribution of uterine blood flow in ovine pregnancy. *American Journal of Obstetrics and Gynecology*, **130**, 385–390.

Rosenfeld, C.R., Worley, R.J. and Gant, N. (1977) Uteroplacental blood flow and oestrogen production following dehydroisoandrosterone infusion. *Obstetrics and Gynecology*, **50**, 304–307.

Rosenfeld, C.R., Worley, R.J., Milewich, L., Gant, N.F. and Parker, C.R. Jr. (1980) Ovine fetoplacental sulfoconjugation and aromatization of dehydroepiandrosterone. *Endocrinology*, **106**, 1971–1979.

Rossdale, P.D. and Silver, M. (1982) The concept of readiness for birth. *Journal of Reproduction and Fertility*, Suppl. 32, 507–510.

Rossdale, P.D., Ousey, J.C., Dudan, F.E., Ledson, D.P. , Cash, R.S.G., Reddy, R., Silver, M., Fowden, A.L., Broughton-Pipkin. F. and Jeffcott, L.B. (1984) Studies on equine prematurity, 1: methodology. *Equine Veterinary Journal*, **16**, 275–278.

Rossdale, P.D., Pashen, R.L. and Jeffcott, L.B. (1979) The use of synthetic prostaglandin analogue (fluprostenol) to induce foaling. *Journal of Reproduction and Fertility*, Suppl. 27, 521–529.

Rossier, G. and Pierrepoint, C.G. (1974) Oestrogen metabolism in sheep myometrium. *Journal of Reproduction and Fertility*, 37, 43–49.

Saeed, S.A., McDonald-Gibson, W.J., Cuthbert, J., Copas, J.L., Schneider, C., Gardiner, P.J., Butt, N.M. and Collier, H.O.J. (1977) Endogenous inhibitor of prostaglandin synthetase. *Nature (London)*, **270**, 32–36.

Saeed, S.A., Strickland, D.M., Young, D.C., Dang, A. and Mitchell, M.D. (1982) Inhibition of prostaglandin synthesis by human amniotic fluid: Acute reduction in inhibitory activity of amniotic fluid obtained during labor. *Journal of*

Clinical Endocrinology and Metabolism, **55**, 801–805.

Saito, Y., Sakamoto, H., McLusky, N.J., Naftolin, F. (1985) Gap junctions and myometrial steroid hormone receptors in pregnant and postpartum rats: a possible cellular basis for the progesterone withdrawal hypothesis. *American Journal of Obstetrics and Gynecology*, **151**, 805–812.

Sakamoto, H., Den, K., Yamamoto, K., Arai, T., Kawai, S., Oyami, Y., Yoshida, T. and Takagi, S. (1979) Study of oxytocin receptor in human myometrium using highly specific ^{3} H-labelled oxytocin. *Endocrinologica Japonica*, **26**, 515–522.

Sakbun, V., Koay, E.S.C. and Bryant-Greenwood, G.D. (1987) Immunocytochemical localization of prolactin and relaxin C-peptide in human decidua and placenta. *Journal of Clinical Endocrinology and Metabolism*, **65**, 339–343.

Saldivar, J.T. and Melton, C.E. (1966) Effects *in vivo* and *in vitro* of sex steroids on rat myometrium. *American Journal of Physiology*, **211**, 835–843.

Sander, H., Bruhn, T.H., Elger, W. and Ellendorff, F. (1982) Prostaglandin E (Sulprostone) is neither luteolytic nor luteotrophic during the estrous cycle in the pig. *Prostaglandins*, **24**, 691–700.

Sanyal, M.K. (1978) Secretion of progesterone during gestation in the rat. *Journal of Endocrinology*, **79**, 179–190.

Sasaki, A., Liotta, A.S., Luckey, M.M., Margioris, A.N., Suda, T. and Krieger, D.T. (1984) Immunoreactive corticotropin-releasing factor is present in human maternal plasma during the third trimester of pregnancy. *Journal of Clinical Endocrinology and Metabolism*, **59**, 812–814.

Schatz, F., Markiewic, L. and Gurpide, E. (1987) Differential effects of estradiol, arachidonic acid and A23187 on prostaglandin F_2 output by epithelial and stromal cells of human endometrium. *Endocrinology*, **120**, 1465–1471.

Schneider, T.M., Tilton, J.E., Okrasa, S., Mah, J., Weigl, R. and Williams, G.L. (1983) The effect of intrauterine infusions of PGE_2 on luteal function in nonpregnant gilts. *Theriogenology*, **20**, 509–520.

Schrams, D., Hoffman, B., Fischer, S., Marz, E. and Karg, H. (1972) Simultaneous determination of LH and progesterone in peripheral bovine blood during pregnancy, normal and corticoid induced parturition and the postpartum period. *Journal of Reproduction and Fertility*, **29**, 37–48.

Schrey, M.P., Read, A.M. and Steer, P.J. (1987) Stimulation of phospholipid hydrolysis and arachidonic acid mobilization in human uterine decidua cells by phorbol ester. *Biochemical Journal*, **246**, 705–713.

Schriefer, J.A., Lewis, P.R. and Miller, J.W. (1982) Role of fetal oxytocin in parturition in the rat. *Biology of Reproduction*, **27**, 362–368.

Schulster, D., Burstein, S. and Cooke, B.A. (1976) *Molecular Endocrinology of the Steroid Hormones*. John Wiley & Sons, London, pp. 1–150.

Schwarz, B.E., Milewich, L., Gant, N.F., Porter, J.C., Johnston, J.M. and MacDonald, P.C. (1977) Progesterone binding and metabolism in human fetal membranes. *Annals of the New York Academy of Science*, **286**, 304–312.

Seamans, K.W., Harms, P.C., Atkins, D.T. and Fleeger, J.L. (1979) Serum levels of progesterone, 5-dihydroprogesterone and hydroxy-5α-pregnanes in the prepartum and postpartum equine. *Steroids*, **33**, 2385–2393.

Seamark, R.F., Nancarrow, C.D. and Gardiner, J. (1970) Progesterone metabolism in ovine blood: the formation of 3α-hydroxy-preg-4-en-20-one and other substances. *Steroids*, **15**, 589–603.

Sellers, S.M., Hodgson, H.T., Mountford, L.A., Mitchell, M.D., Anderson, A.B.M. and Turnbull, A.C. (1981) Is oxytocin involved in parturition? *British Journal of Obstetrics and Gynaecology*, **88**, 725–729.

Seren, E., Tamanini, C., Gaiani, R. and Bono, G. (1981) Concentrations of progesterone, 17α-hydroxyprogesterone, and 20α-dihydroprogesterone in the plasma of mares during pregnancy and at parturition. *Journal of Reproduction and Fertility*, **63**, 443–448.

Shaik, A.A. (1971) Estrone and estradiol levels in the ovarian venous blood from rats during estrous cycle and pregnancy. *Biology of Reproduction*, **5**, 297–307.

Sheldrick, E.L. and Flint, A.P.F. (1986) Transient uterine refractoriness after oxytocin administration in ewes. *Journal of Reproduction and Fertility*, **77**, 523–529.

Sheldrick, E.L., Ricketts, A.P. and Flint, A.P.F. (1980) Placental production of progesterone in ovariectomized goats treated with a synthetic progestagen to maintain pregnancy. *Journal of Reproduction and Fertility*, **60**, 339–348.

Sheldrick, E.L., Ricketts, A.P. and Flint, A.P.F. (1981) Placental production of 5β-pregnane-3α,20α-diol in goats. *Journal of Endocrinology*, **90**, 151–158.

Shemesh, M., Hansel, W. and Strauss, J.F. (1984a) Modulation of bovine placental prostaglandin synthesis by an endogenous inhibitor. *Endocrinology*, **115**, 1401–1405.

Shemesh, M., Hansel, W. and Strauss, J.F. (1984b) Calcium-dependent, cyclic nucleotide-independent steroidogenesis in the bovine placenta. *Animal Reproductive Science*, **7**, 177–194.

Sherwood, O.D., Chang, C.C., BeVier, G.W. and Dziuk, P.J. (1975) Radioimmunoassay of plasma relaxin levels throughout pregnancy and at parturition in the pig. *Endocrinology*, **97**, 834–837.

Sherwood, O.D., Chang, C.C., BeVier, G.W., Diehl, J.R. and Dziuk, P.J. (1976) Relaxin concentration in pig plasma following the administration of prostaglandin $F_{2\alpha}$ during late pregnancy. *Endocrinology*, **98**, 875–879.

Sherwood, O.D., Wilson, M.E., Edgerton, L.A. and Chang, C.C. (1978) Serum relaxin concentrations in pigs with parturition delayed by progesterone administration. *Endocrinology*, **102**, 471–475.

Sherwood, O.D., Nara, B.S., Crnekovic, V.E. and First, N.L. (1979) Relaxin concentrations in pig plasma after the administration of indomethacin and prostaglandin $F_{2\alpha}$ during late pregnancy. *Endocrinology*, **104**, 1716–1721.

Sherwood, O.D., Crnekovic, V.E., Gordon, W.L. and Rutherford, J.E. (1980) Radioimmunoassay of relaxin throughout pregnancy and during parturition in the rat. *Endocrinology*, **107**, 691–698.

Sherwood, O.D., Nara, B.S., Welk, F.A., First, N.L. and Rutherford, J.E. (1981) Relaxin levels in the maternal plasma of pigs before, during and after parturition and before, during and after suckling. *Biology of Reproduction*, **25**, 65–71.

Shinada, T., Yokota, Y. and Igarashi, M. (1978) Inhibitory effect of various gestagens upon the pregnenolone 3β-ol-dehydrogenase $\delta_{5,4}$-isomerase system in human corpora lutea of menstrual cycles. *Fertility and Sterility*, **29**, 84–87.

Short, R.E., James, L.F., Staigmiller, R.B. and Panter, R.E. (1989) Pine needle abortion in cattle; associated changes in serum cortisol, estradiol and progesterone. *Cornell Veterinarian*, **79**, 53–60.

Short, R.V. (1958) Progesterone in the blood. II. Progesterone in the peripheral blood of pregnant cows. *Journal of Endocrinology*, **16**, 426–428

Short, R.V. and Moore, N.W. (1959) Progesterone in blood. V. Progesterone and 20α-hydroxypregn-4-en-3-one in the placenta and blood of ewes. *Journal of Endocrinology*, **19**, 288–293.

Silver, M. and Fowden, A.L. (1982) Uterine prostaglandin F metabolite production in relation to glucose availability in late pregnancy and a possible influence of diet on time of delivery in the mare. *Journal of Reproduction and Fertility*, Suppl. 32, 511–519.

Silver, M. and Fowden, A.L. (1989) Pituitary–adrenocortical activity in the fetal pig in the last third of gestation. *Quarterly Journal of Experimental Physiology*, **74**, 197–206.

Silver, M., Barnes, R.J., Comline, R.S., Fowden, A.L., Clover, L. and Mitchell, M.D. (1979a) Prostaglandins in the foetal pig and prepartum changes in the mother and fetus. *Animal Reproduction Science*, **2**, 305–322.

Silver, M., Barnes, R.J., Comline, R.S., Fowden, A.L., Clover, L. and Mitchell, M.D. (1979b) Prostaglandins in maternal and fetal plasma and in allantoic fluid during the second half of gestation in the mare. *Journal of Reproduction and Fertility*, Suppl. 27, 531–539.

Silver, M., Comline, R.S. and Fowden, A.L. (1983) Fetal and maternal endocrine changes during the induction of parturition with the PGF analogue, cloprostenol, in chronically catheterized sows and fetuses. *Journal of Developmental Physiology*, **5**, 307–321.

Simpson, E.R. (1984) Lipoproteins and steroidogenesis in the human corpus luteum and placenta, in, *Initiation of Parturition: Prevention of Prematurity*. Ross Conference, Dallas, Texas, USA, Ross Laboratories, pp. 94–103.

Singh, M.M. and Adams, C.E. (1978) Luteotrophic effect of the rabbit blastocyst. *Journal of Reproduction and Fertility*, **53**, 331–333.

Skinner, K.A. and Challis, J.R.G. (1985) Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. *American Journal of Obstetrics and Gynecology*, **151**, 519–523.

Skinner, S.J.M., Liggins, G.C., Wilson, T. and Neale, G. (1984) Synthesis of prostaglandin F by cultured human endometrial cells. *Prostaglandins*, **27**, 821–828.

Small, M.G.P., Gavagan, J.E. and Roberts, J.S. (1978) Oxytocin-stimulated production of prostaglandin $F_{2\alpha}$ by isolated endometrium of rabbit: Modulation by ovarian steroids. *Prostaglandins*, **15**, 103–112.

Smith, P.E. (1954) Continuation of pregnancy in

rhesus monkeys (*Macaca mulatta*) following hypophysectomy. *Endocrinology*, **55**, 655–664.

Sokolowski, J.H. (1971) The effects of ovariectomy on pregnancy maintenance in the bitch. *Laboratory Animal Science*, **21**, 696–699.

Soloff, M.S. (1975) Uterine receptor for oxytocin: effects of estrogen. *Biochemical and Biophysical Research Communications*, **65**, 205–212.

Soloff, M.S. and Swartz, T.L. (1974) Characterization of a proposed oxytocin receptor in the uterus of the rat and sow. *Journal of Biological Chemistry*, **249**, 1376–1381.

Soloff, M.S., Swartz, T.L. and Steinberg, A.H. (1974) Oxytocin receptors in human uterus. *Journal of Clinical Endocrinology and Metabolism*, **38**, 822–826.

Soloff, M.S., Fernstrom, M.A., Periyasamy, S., Soloff, S., Baldwin, S. and Weider, M. (1983) Regulation of oxytocin receptor concentration in rat uterine explants by estrogen and progesterone. *Canadian Journal of Biochemistry and Cell Biology*, **61**, 625–630.

Speroff, L. and Ramwell, P.W. (1970) Prostaglandin stimulation of *in vitro* progesterone synthesis. *Journal of Clinical Endocrinology and Metabolism*, **30**, 345–350.

Spies, H.G., Slyter, A.L. and Quadri, S.K. (1967) Regression of corpora lutea in pregnant gilts administered antiovine LH rabbit serum. *Journal of Animal Science*, **26**, 768–771.

Stabenfeldt, G.H. and Hendrickx, A.G. (1972) Progesterone levels in the bonnet monkey (*Macaca radiata*) during the menstrual cycle and pregnancy. *Endocrinology*, **91**, 614–619.

Stabenfeldt, G.H. and Hendrickx, A.G. (1973a) Progesterone studies in the Macaca fascicularis. *Endocrinology*, **92**, 1296–1300.

Stabenfeldt, G.H. and Hendrickx, A.G. (1973b) Progesterone levels in the Sooty mangabey (*Cercocebus atys*) during the menstrual cycle, pregnancy and parturition. *Journal of Medical Primatology*, **2**, 1–10.

Stabenfeldt, G.H., Osburn, B.I. and Ewing, L.L. (1970) Peripheral plasma progesterone levels in the cow during pregnancy and parturition. *American Journal of Physiology*, **218**, 571–576.

Stabenfeldt, G.H., Drost, M. and Franti, C.E. (1972) Peripheral progesterone levels in the ewe during pregnancy and parturition. *Endocrinology*, **90**, 144–150.

Stanczyk, F.Z., Hess, D.L., Namkung, P., Senner, J.W., Petra, P.H. and Novy, M.J. (1986) Alterations in sex steroid-binding protein, CBG and steroid hormone concentrations during pregnancy in rhesus macaques. *Biology of Reproduction*, **35**, 126–132.

Steele, P.A., Flint, A.P. and Turnbull, A.C. (1976) Activity of steroid C-17,20 lyase in the ovine placenta: effect of exposure to foetal glucocorticoid. *Journal of Endocrinology*, **69**, 239–246.

Steinetz, B.G., Goldsmith, L.T., Harvey, H.J. and Lust, G. (1989) Serum relaxin and progesterone concentrations in pregnant, pseudopregnant, and ovariectomized, progestin-treated pregnant bitches: detection of relaxin as a marker of pregnancy. *American Journal of Veterinary Research*, **50**, 68–71.

Steven, D.H. (1977) Ultrastructure of the sheep placenta, in *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Symposium No. 47, Elsevier, Amsterdam, pp. 99–10

Stewart, D.R., Stabenfeldt, G.H. and Hughes, J.P. (1982) Relaxin activity in foaling mares. *Journal of Reproduction and Fertility*, Suppl. 32, 603–609.

Stewart, D.R., Kindahl, H., Stabenfeldt, G.H. and Hughes, J.P. (1984) Concentrations of 15-keto-13,14-dihydro-prostaglandin F_2 alpha in the mare during spontaneous and oxytocin induced foaling. *Equine Veterinary Journal*, **16**, 270–274.

Strickland, D.M., Saeed, S.A., Casey, M.L. and Mitchell, M.D. (1983) Stimulation of prostaglandin biosynthesis by urine of the human fetus may serve as a trigger for parturition. *Science*, **220**, 521–522.

Strott, C.A., Sundel, H. and Stahlman, M.T. (1974) Maternal and fetal plasma progesterone, cortisol, testosterone and 17β-estradiol in preparturient sheep: response to fetal ACTH infusion. *Endocrinology*, **95**, 1327–1339.

Stryker, J. and Dziuk, P.J. (1975) Effects of fetal decapitation on fetal development, parturition and lactation in pigs. *Journal of Animal Science*, **40**, 282–287.

Sumida, C., Lecerf, F. and Pasqualini, J.R. (1988) Control of progesterone receptors in fetal uterine cells in culture: Effects of estradiol, progestins, antiestrogens, and growth factors. *Endocrinology*, **122**, 3–11.

Sunde, A., Rosness, P.A. and Eik-Nes, K.B. (1982) Effects *in vitro* of medroxyprogesterone acetate on steroid metabolizing enzymes in the rat: selective inhibition of 3α-hydroxysteroid oxidoreductase activity. *Journal of Steroid Biochemistry*, **17**, 197–203.

Swaab, D.F. and Oosterbaan, H.P. (1983) Exclusion of the fetal brain as the main source of

rat and human amniotic fluid oxytocin. *British Journal of Obstetrics and Gynaecology*, **90**, 1160–1167.

Swaab, D.F., Boer, K. and Honnebier, W.J. (1977) The influence of the fetal hypothalamus and pituitary on the onset and course of parturition, in *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Symposium No. 47, Elsevier, Amsterdam, pp. 379–393.

Takahashi, M., Shiota, K. and Suzuki, Y. (1979) Introduction of a new crop of corpora lutea during pregnancy and its effects on luteal function and parturition in the rat. *Biology of Reproduction*, **21**, 813–822.

Takeishi, M., Shibata, S. and Tsumagari, S. (1989) Adrenocorticotropin and cortisol levels in the plasma of bovine fetuses and neonates. *Nippon Juigaku Zasshi*, **51**, 975–980.

Taverne, M. (1982) Myometrial activity during pregnancy and parturition, in, *Control of Pig Reproduction*, (eds D.J.A. Cole and G.R. Foxcroft), Butterworths, London, pp. 419–436.

Taverne, M., Willemse, A.H., Dieleman, S.J. and Bevers, M. (1979a) Plasma prolactin, progesterone and oestradiol-17β concentrations around parturition in the pig. *Animal Reproduction Science*, **1**, 257–263.

Taverne, M.A.M., Naaktgeboren, C., Elsaesser, F., Forsling, M.L., van der Weyden, G.C., Ellendorf, F. and Smidt, D. (1979b) Myometrial electrical activity and plasma concentrations of progesterone, oestrogen and oxytocin during late pregnancy and parturition in the miniature pig. *Biology of Reproduction*, **21**, 1125–1134.

Taverne, M., Bevers, M., Bradshaw, J.M.C., Dieleman, S.J., Willemse, A.H. and Porter, D.G. (1982) Plasma concentrations of prolactin, progesterone, relaxin and oestradiol-17β in sows treated with progesterone, bromocriptine or indomethacin during late pregnancy. *Journal of Reproduction and Fertility*, **65**, 85–96.

Taya, K. and Greenwald, G.S. (1981) *In vivo* and *in vitro* ovarian steroidogenesis in the pregnant rat. *Endocrinology*, **108**, 683–691.

Taylor, M.J., Webb, R., Mitchell, M.D. and Robinson, J.S. (1982) Effect of progesterone withdrawal in sheep during late pregnancy. *Journal of Endocrinology*, **92**, 85–93.

Taylor, M.J., McMillen, I.C., Jenkin, G., Robinson, J.S. and Thorburn, G.D. (1983) The regulation of ovine placental lactogen: the role of the fetal hypothalamic–pituitary axis. *Journal of Developmental Physiology*, **5**, 251–258.

Tetta, G., Montrucchio, G., Alloatti, G., Roffinello, C., Emanuelli, G., Benedetto, C., Camussi, G. and Massobrio, M. (1986) Platelet-activating factor contracts human myometrium *in vitro*. *Proceedings of the Society of Experimental Biology and Medicine*, **183**, 376–381.

Thau, R. and Lanman, J.T. (1974) Evaluation of progesterone synthesis in rabbit placentas. *Endocrinology*, **94**, 925–926.

Theobald, G.W. (1970) The neurohypophysis in labour, in, *Scientific Foundations of Obstetrics and Gynaecology*, (eds E.E. Philip, F. Barnes and M. Newton), William Heinemann, London, pp. 514–530.

Thorburn, G.D. (1977) The fetus, pregnancy and parturition. Proceedings of the International Congress of Physiological Scientists, Lyons, France. *Annales des Recherches Veterinaires*, **8**, 428–437.

Thorburn, G.D. (1978) Hormonal control of parturition in the sheep and goat. *Seminars in Perinatology*, **2**, 235–245.

Thorburn, G.D. (1979) Physiology and control of parturition: reflections on the past and ideas for the future. *Animal Reproduction Science*, **2**, 1–27.

Thorburn, G.D. (1983) Past and present concepts of the initiation of parturition, in, *Initiation of Parturition: Prevention of Prematurity*, (eds P.C. MacDonald and J. Porter) 4th Ross Conference on Obstetric Research, Ross Laboratories, Columbus, Ohio, pp. 2–11.

Thorburn, G.D. (1991) The placenta, prostaglandins and parturition. 1989 Goding Lecture, ASRB/ESA/NZSE Conference, September, Monash University, Melbourne, Australia. *Reproduction Fertility and Development*, **3**, 277–294.

Thorburn, G.D. (1993) A speculative review of parturition in the mare. *Equine Veterinary Journal*, Suppl. 14, 41–49.

Thorburn, G.D. and Challis, J.R.G. (1979) Endocrine control of parturition. *Physiological Reviews*, **59**, 863–917.

Thorburn, G.D. and Nicol, D.H. (1971) Regression of the ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into the ovarian artery and uterine vein. *Journal of Endocrinology*, **51**, 785–786.

Thorburn, G.D. and Rice, G.E. (1990) Placental PGE_2 and the initiation of parturition in sheep, in, *Eicosanoids in Reproduction*, (ed. M.D. Mitchell) CRC Press, Boca Raton, Florida, pp. 73–86.

Thorburn, G.D. and Schneider, W. (1972) The progesterone concentration in the plasma of the goat during the oestrus cycle and pregnancy. *Journal of Endocrinology*, **52**, 23–36.

Thorburn, G.D., Nicol, D.H., Bassett, J.M., Shutt, D.A. and Cox, R.I. (1972a) Parturition in the goat and sheep: changes in corticosteroids, progesterone, oestrogens and prostaglandin F. *Journal of Reproduction and Fertility*, Suppl. 16, 61–84.

Thorburn, G.D., Cox, R.I., Currie, W.B., Restall, B.J. and Schneider, W. (1972b) Prostaglandin F concentration in the utero-ovarian venous plasma of the ewe during the oestrous cycle. *Journal of Endocrinology*, **53**, 325–326.

Thorburn, G.D., Challis, J.R.G. and Robinson, J.S. (1977a) Endocrine control of parturition, in, *Biology of the Uterus*, (ed. R.M. Wynn) Plenum Press, New York, 653–732.

Thorburn, G.D., Challis, J.R.G. and Currie, W.B. (1977b) Control of parturition in domestic animals. Invited lecture, Society for the Study of Reproduction, Philadelphia, USA. *Biology of Reproduction*, **16**, 18–27.

Thorburn, G.D., Jenkin, G., Harding, R., Parkington, H. and Sigger, J.N. (1983) Progesterone, prostaglandin $F_{2\alpha}$ and parturition in the sheep, in, *Endocrinology*, (eds K. Shizume, H. Imura and N. Shimuza) Proceedings of the 7th Asian and Oceania Congress of Endocrinology, Tokyo, Japan, Excerpta Medica, Amsterdam, pp. 376–380.

Thorburn, G.D., Harding, R., Parkington, H., Sigger, J.N. and Jenkin, G. (1984) Control of uterine activity in the sheep. *Journal of Developmental Physiology*, **6**, 31–43.

Thorburn, G.D., Hooper, S.B., Rice, G.E. and Fowden, A.L. (1988) Luteal regression and parturition: a comparison, in, *Cellular and Integrative Mechanisms in the Onset of Labor – What are the Research Priorities*? (eds D. McNellis, J.R.G. Challis, P. MacDonald, P. Nathanielsz and J. Roberts) NICHD Workshop, Bethesda, Perinatology Press, Ithaca, pp. 185–210.

Thorburn, G.D., Deayton, J.M., Young, I.R. and Ralph, M.M. (1989) The effects of early fetal hypophysectomy on ovine placental PGE_2 and steroid biosynthesis. *Journal of Reproduction and Fertility*, Abstract Series No. 3 ,Abstract 49.

Thorburn, G.D., Hollingworth, S.A. and Hooper, S.B. (1991) The trigger for parturition in sheep: fetal hypothalamus or placenta? *Journal of Developmental Physiology*, **15**, 71–79.

Torii, R., Nigi, H., Koizumi, H. and Tanioka, Y. (1989) Serum chorionic gonadotropin, progesterone, and estradiol-17β during pregnancy in the common marmoset, *Callithrix jacchus*. *Primates*, **30**, 207–215.

Tsang, C.P. and Hackett, A.J. (1979) Metabolism of progesterone in the pregnant sheep near term: identification of 3 beta-hydroxy-5 alpha-pregnan-20-one-3-sulfate as a major metabolite. *Steroids*, **33**, 577–588.

Turnbull, A.C. and Anderson, A.B.M. (1968) Uterine contractility and oxytocin sensitivity during human pregnancy in relation to the onset of labour. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **75**, 278–288.

Turnbull, A., Flint, A., Jeremy, J., Patten, P., Keirse, M. and Anderson, A. (1974) Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before the onset of labour. *Lancet*, **1**, 101–104.

Turnbull, A.C., Anderson, A.B.M., Flint, A.P.F., Jeremy, J.Y., Keirse, M.J.N.C. and Mitchell, M.D. (1977) Human parturition. In *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Symposium No. 47, Elsevier, Amsterdam, pp. 427–452.

Tyson, J.E., Dubin, N. and McCoshen, J.A. (1985) Inhibition of fetal membrane production by prolactin: relative importance in the initiation of labour. *American Journal of Obstetrics and Gynecology*, **151**, 1032–1038.

Umo, I., Fitzpatrick, R.J. and Ward, W.R. (1976) Parturition in the goat: plasma concentrations of prostaglandin F and steroid hormones and uterine activity during late pregnancy and parturition. *Journal of Endocrinology*, **68**, 383–389.

Vallet, J.L., Lamming, G.E. and Batten, M. (1990) Effect of ovine conceptus secretory proteins and bovine recombinant interferonα-1 on endometrial oxytocin receptor concentrations and uterine response to oxytocin in cyclic and progesterone treated ovariectomised ewes. *Journal of Reproduction and Fertility*, Abstract Series No. 4, Abstract 17.

Van Leusden, H. and Villee, C.A. (1965) The *de novo* synthesis of sterols and steroids from acetate by preparations of human term placenta. *Steroids*, **6**, 31–45.

Vane, J.R. (1978) Inhibitors of prostaglandin, prostacyclin and thromboxane synthesis, in, *Advances in Prostaglandin and Thromboxane Research*, (eds F. Coceani and P. Olley) New York, Raven Press, Vol. 4, pp. 27–44.

Van Wagenen, G. and Newton, W.H. (1943) Pregnancy in the monkey after removal of the fetus. *Surgery Gynecology and Obstetrics*, **77**, 539–546.

Veldhuis, J.D., Klase, P.A. and Hammond, J.M. (1981) Direct actions of 17β-estradiol on progesterone production by highly differentiated porcine granulosa cells *in vitro*. II. Regulatory interactions of estradiol with luteinizing hormone and cyclic nucleotides. *Endocrinology*, **109**, 433–442.

Velle, W. (1958) Investigations on naturally occurring oestrogens in ruminants and the pig. *PhD Thesis*. The Veterinary College of Norway, Oslo.

Wagner, G. (1974) Contractility of myometrial transplants, in, *Physiology and Genetics of Reproduction*, (eds E.M. Coutinho and F. Fuchs), Part B. Plenum Press, New York, pp. 203–218.

Wallner, B.P., Mattallano, R.J., Hesslon, C., Cate, R.L., Tizard, R., Sinclair, L.K., Foeller, C., Chow, E.P., Browning, J.L. and Ramachandran, K.L. (1986) Cloning and expression of human lipocortin, a phospholipase A_2 inhibitor with potential anti-inflammatory activity. *Nature (London)*, **320**, 77–81.

Walsh, S.W. (1991) 5-hydroxyeicosatetraenoic acid, leukotriene C_4 , and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor. *Annals of the New York Academy of Science*, **622**, 341–354.

Walsh, S.W., Meyer, R.K., Wolf, R.C. and Friesen, H.G. (1977a) Corpus luteum and fetoplacental functions in monkeys hypophysectomised during late pregnancy. *Endocrinology*, **100**, 845–850.

Walsh, S.W., Wolf, R.C., Meyer, R.K., Aubert, M.L. and Friesen, H.M. (1977b) Chorionic gonadotropin, chorionic somatomammotropin, and prolactin in the uterine vein and peripheral plasma of pregnant rhesus monkeys. *Endocrinology*, **100**, 851–855.

Walsh, S.W., Kittinger, G.W. and Novy, M.J. (1979) Maternal peripheral concentrations of estradiol, cortisol, and progesterone during late pregnancy in rhesus monkeys (*Maccaca mulatta*) and after experimental fetal anencephaly and fetal death. *American Journal of Obstetrics and Gynecology*, **135**, 37–42.

Walsh, S.W., Resko, J.A., Grumbach, M.M. and Novy, M.J. (1980) In utero evidence for a functional fetoplacental unit in rhesus monkeys. *Biology of Reproduction*, **23**, 264–270.

Walsh, S.W., Ducsay, C.A. and Novy, M.J. (1984) Circadian hormonal interactions among the mother, fetus and amniotic fluid. *American Journal of Obstetrics and Gynecology*, **150**, 745–751.

Wang, D.Y., Bulbrook, R.D., Sneddon, A. and Hamilton, T. (1967) The metabolic clearance rates of dehydroepiandrosterone, testosterone and their sulphate esters in man, rat and rabbit. *Journal of Endocrinology*, **38**, 307–318.

Wango, E., Wooding, F.B.P. and Heap, R.B. (1988) Differences in the role for prostaglandin E_2 in steroid synthesis of isolated binucleate cells from sheep and goat placenta (abstract). *Journal of Endocrinology*, Suppl. 117, 131.

Ward, W.R. (1968) Structure and function of the ovine cervix uteri. *PhD Thesis*, University of Liverpool, Liverpool.

Warshaw, M.L., Johnson, D.C., Khan, I., Eckstein, B. and Gibori, G. (1986) Placental secretion of androgens in the rat. *Endocrinology*, **119**, 2642–2648.

Watts, A.D., Flint, A.P.F., Foxcroft, G.R. and Porter, D.G. (1988) Plasma steroid, relaxin and dihydro-keto-prostaglandin $F_{2\alpha}$ changes in the minipig in relation to myometrial electrical and mechanical activity in the prepartum period. *Journal of Reproduction and Fertility*, **83**, 553–564.

Webel, S.K., Reimers, T.J. and Dziuk, P.J. (1975) The lack of relationship between plasma progesterone levels and number of embryos and their survival in the pig. *Biology of Reproduction*, **13**, 177–186.

Weiss, G., Steinetz, B.G., Diershke, D.J. and Fritz, G. (1981) Relaxin secretion in the rhesus monkey. *Biology of Reproduction*, **24**, 565–567.

Weist, W.G. (1970) Progesterone and 20α-hydroxypregn-4-en-3-one in plasma, ovaries and uteri during pregnancy in the rat. *Endocrinology*, **87**, 43–48.

Weist, W.G., Kidwell, W.R. and Balogh, K. (1968) Progesterone catabolism in the rat ovary: A regulatory mechanism for progestational potency during pregnancy. *Endocrinology*, **82**, 844–859.

Welch, R.A.S., Frost, O.L. and Bergman, M. (1973) The effect of administering ACTH directly to the fetal calf. *New Zealand Medical Journal*, **78**, 365.

Wendorf, G.L., Lawyer, M.S. and First, N.L. (1983) Role of the adrenals in the maintenance of pregnancy in cows. *Journal of Reproduction and Fertility* 68, 281-287.

Whitely, J.L., Hartmann, P.E., Willcox, D.L., Bryant-Greenwood, G.D. and Greenwood, F.C.

(1990) Initiation of parturition and lactation in the sow: effects of delaying parturition with medroxyprogesterone acetate. *Journal of Endocrinology*, **124**, 475–484.

Willman, E.A. and Collins, W.P. (1976) Distribution of prostaglandin E_2 and $F_{2\alpha}$ within the foetoplacental unit throughout human pregnancy. *Journal of Endocrinology*, **69**, 413–419.

Wilson, L. and Freinkel, N. (1982) Alterations in uterine and placental prostaglandin F and E with gestational age in the rat. *Prostaglandins*, **24**, 567–573.

Wilson, L. and Huang, L.S. (1984) Temporal response of uterine prostaglandins to estradiol treatment in the ovariectomized-pregnant rat. *Prostaglandins*, **28**, 103–110.

Wilson, L. and Lindsey, R. (1987) Effects of progesterone withdrawal on uterine prostaglandin levels in the ovariectomized pregnant rat. *Prostaglandins, Leukotrienes and Medicine*, **29**, 95–105.

Wilson, T., Liggins, G.C., Aimer, G.P. and Skinner, S.J.M. (1985) Partial purification and characterisation of two compounds from amniotic fluid which inhibit phospholipase activity in human endometrial cells. *Biochemical and Biophysical Research Communications*, **131**, 22–29.

Wilson, T., Liggins, G.C., Aimer, G.P. and Watkins, E.J. (1986) The effect of progesterone on the release of arachidonic acid from human endometrial cells stimulated by histamine. *Prostaglandins*, **31**, 343–360.

Wilson, T., Liggins, G.C. and Whittaker, D.J. (1988) Oxytocin stimulates the release of arachidonic acid and prostaglandin $F_{2\alpha}$ from human decidual cells. *Prostaglandins*, **35**, 771–781.

Wilson, T., Liggins, G.C. and Joe, L. (1989) Purification from incubated chorion of a phospholipase A_2 inhibitor active before but not after the onset of labour. *American Journal of Obstetrics and Gynecology*, **160**, 602–606.

Wolf, J.P., Sinosich, M., Anderson, T.L., Ulmann, A., Baulieu, E.E. and Hodgen, G.H. (1989) Progesterone antagonist cervical dilation, labor induction, and delivery in monkeys, Effectiveness in combination with oxytocin. *American Journal of Obstetrics and Gynecology*, **160**, 45–47.

Wooding, F.B.P. (1981) Localization of ovine placental lactogen in sheep placentomes by electron microscope immunocytochemistry. *Journal of Reproduction and Fertility*, **62**, 15–19.

Wooding, F.B.P. and Wathes, D.C. (1980) Binucleate cell migration in the bovine placentome. *Journal of Reproduction and Fertility*, **59**, 425–430.

Yki-Jarvinen, H. and Wahlstrom, T. (1984) Immunohistochemical demonstration of relaxin in placenta after removal of the corpus luteum. *Acta Endocrinologica*, **106**, 544–547.

Yoshinaga, K. (1961) Effect of local application of ovarian hormones on the delay in implantation in lactating rats. *Journal of Reproduction and Fertility*, **2**, 35–41.

Yoshinaga, K. and Hosi, T. (1958) On the delayed implantation in the lactating pregnant rat. *Japanese Journal of Animal Reproduction*, **3**, 393–399.

Yu, H.K., Cabalum, T., Jansen, C.A., Buster, J.E. and Nathanielsz, P.W. (1983) Androstenedione, testosterone and estradiol concentrations in fetal and maternal plasma in late pregnancy in the sheep. *Endocrinology*, **113**, 2216–2220.

Zakar, T. and Olson, D.M. (1988) Stimulation of human amnion prostaglandin E_2 production by activators of protein kinase C. *Journal of Clinical Endocrinology and Metabolism*, **67**, 915–923.

Zakar, T. and Olson, D.M. (1989) Dexamethasone stimulates arachidonic acid conversion to prostaglandin E_2 in human amnion cells. *Journal of Developmental Physiology*, **12**, 269–272.

Ziecik, A., Shaw, J.H. and Flint, A.P.F. (1980) Luteal LH receptors during the oestrous cycle and early pregnancy in the pig. *Journal of Reproduction and Fertility*, **60**, 129–137.

ROLE OF THE FETAL PITUITARY–ADRENAL AXIS AND PLACENTA IN THE INITIATION OF PARTURITION

10

G.D. Thorburn and G.C. Liggins

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10.1 HISTORICAL ASPECTS

The concept that the fetus initiates its own delivery dates from 460 BC, when Hippocrates suggested that, at the appointed hour, the fetus puts its feet on the fundus of the uterus and pushes to effect its own delivery. It was not until the tenth century that the Italian anatomist, Fabricius ab Aquapendente, proposed that the chief agent in the initiation of parturition was the muscular action of the uterus. In 1882, Spiegelberg re-emphasized the role of the fetus when he wrote in a German textbook of midwifery that, '. . . the reason why labour occurs at a definite time must be sought for, not in the uterus and its changes, but in the fetus. It is the **maturity** of the latter that gives the signal.'. In 1933, Malpas reported a series of cases of prolonged pregnancy associated with fetal anencephaly. Malpas concluded that 'the time of the onset of labour is determined by the foetus', and 'the foetal adrenal, pituitary, or nervous system, perhaps in combination, are suggested as tissues possibly concerned in the actual excitation of the neuromuscular expulsive mechanisms'.

Prolonged pregnancy in cattle has long been recognized as a clinical entity. Most inbred breeds of dairy cattle produce genetically defective calves. Investigation of affected fetuses provided the first definitive evidence for the fetal pituitary–adrenal axis playing a primary role in the initiation of parturition. Prolonged gestation in Holstein–Friesian (Jasper, 1950) and Guernsey (Kennedy *et al.*, 1957) breeds was due to a homozygous recessive autosomal gene in the fetus that resulted in fetal endocrinopathies (reviewed by Holm, 1967). In the cows carrying affected fetuses, the corpus luteum (CL) of pregnancy did not regress, and, rather than decreasing at term, progesterone (P_4) concentrations were maintained throughout gestation (Holm and Short, 1962). The affected calves of the Guernsey breed were premature in development, failing to grow beyond the size of a 7-month fetus, and always exhibited adenohypophyseal aplasia. Although the adrenal cortex and medulla were distinguishable, the adrenal glands were small and the cortical architecture was undeveloped. Gestation in these animals ranged from 292 to 526 days (normal term, 280 days). The affected calves of the Holstein–Friesian breed continued to grow during their prolonged gestation and were unusually large, but they failed to initiate labour and died *in utero* about 100 days past term (Holm, 1967). The adenohypophysis, although present, was smaller than normal (Holm, 1967).

Naturally occurring prolonged gestation has also been observed in sheep. In 1963, a congenital cyclopian-type malformation associated with prolonged pregnancy was identified in fetuses of range sheep in Idaho (Binns *et al.*, 1963, 1964). The teratogenic effect resulted from the ingestion of skunk cabbage (*Veratrum californicum*) on the fourteenth day of gestation. The toxic principle was 11-deoxyjervine, a steroidal alkaloid. The affected lambs had pituitary glands, although the neural connections were either missing or abnormal (Kennedy, 1971). In 1969, Basson *et al.* reported cases of prolonged pregnancy in sheep due to the ingestion of the shrub *Salsola tuberculata* during the second half of gestation: gestation was prolonged for about 2 weeks, and at delivery the lambs were found to have atrophic adrenal cortices.

Clinical syndromes of non-infectious cause characterized by premature delivery are rare. The syndrome of recurrent abortion in highly inbred Angora goats has been studied by van Rensburg (1971), who demonstrated fetal adrenal hyperplasia in the aborted fetuses and suggested that premature activation of the fetal pituitary–adrenal axis may be responsible for the abortion.

The above observations provided evidence which involved the fetus in the aetiology of prolonged pregnancy although the data indi-

cating the specific involvement of the fetal pituitary–adrenal axis were less convincing. Liggins *et al.* (1967) established that, after electrocoagulation of the pituitary of an otherwise normal fetal lamb, pregnancy was prolonged indefinitely and was associated with hypoplasia of the adrenal cortices (Liggins and Kennedy, 1968). Parturition occurred at the expected time in multiple pregnancies if one fetus had an intact pituitary, or in single pregnancies if less than 70% of the pituitary tissue was removed. Prolonged pregnancy was also reported in two ewes after bilateral fetal adrenalectomy (Drost and Holm, 1968). Ablation of the fetal adrenal medullary tissue did not result in prolonged gestation, indicating the primary role of the adrenal cortex (Liggins, 1969a).

These experiments clearly established an essential role for the fetal pituitary in the initiation of parturition but did not determine whether the fetus played an active or passive role in the process. Liggins (1969b) showed that fetal infusion of synthetic $ACTH_{1-24}$, but not other pituitary hormones, caused premature delivery in 4–7 days. The infusion of glucocorticoid hormones, such as cortisol and dexamethasone, into the fetal lamb also induced premature labour. Infusion of these compounds at similar doses per unit weight into the mother were ineffective in influencing the length of gestation. Furthermore, the active principle appeared to reside in the glucocorticoid moiety because infusions of mineralocorticoids such as deoxycorticosterone were ineffective (Liggins, 1968, 1969b).

These experiments showed that an intact fetal pituitary–adrenal axis was essential for the initiation of parturition in sheep and that activation of this pathway could cause premature labour. Bassett and Thorburn (1969) provided evidence that activation of the fetal pituitary–adrenal axis preceded normal-term parturition. In the chronically catheterized sheep fetus, there is a gradual increase in plasma glucocorticoids beginning about 15 days before term, accelerating to a marked increase in the last 3–4 days, a finding consistent with the earlier observation of Comline and Silver (1961) that the adrenal glands of the fetal lamb grow markedly in the last 10 days or so of intrauterine life. However, these findings gave no insight into the trigger mechanism which activated the fetal pituitary–adrenal axis. (HPA; abbreviations used in this chapter are given in Table 10.1).

Table 10.1 Abbreviations used in chapter 10

$17\text{-}OHP_4$	17α-hydroxyprogesterone
$17\text{-}OHP_5$	17α-hydroxypregnolone
ACTH	adrenocorticotrophic hormone
AVP	arginine vasopressin
B	corticosterone
CBG	corticosteroid-binding globulin
CRF	corticotrophin-releasing factor
EP	receptor for PGE_2
F	cortisol
Gpp(NH)p	guanylylimidodiphosphate
GTP	guanine triphosphate
HETE	eicosatetranoic acid
HMW	high molecular weight
HPA	hypothalamo – pituitary – adrenal
HPD	hypothalamo – pituitary disconnected
HPETE	hydroperoxyeicosatetraenoic acid
IP	inositol phosphate
IRMA	immunoradiometric assay
βLPH	β-lipotrophin
ME	medium eminence
MSH	melanocyte-stimulating hormone
NDGA	nordihydroguaiaretic acid
P_4	progesterone
P_5	pregnenolone
PGE_2	prostaglandin E_2
POMC	proopiomelanocortin
PVN	paraventricular nucleus
RIA	radioimmunoassay
SON	supraoptic nucleus
TGF	transforming growth factor

10.2 PLASMA CORTISOL CONCENTRATIONS IN THE FETAL LAMB

The preparturient increase in fetal plasma glucocorticoids concentration first described

by Bassett and Thorburn (1969, 1973) has been reconfirmed many times (Comline *et al.*, 1970; Alexander *et al.*, 1973; Drost *et al.*, 1973; Strott *et al.*, 1974; Thomas *et al.*, 1976; Brown *et al.*, 1978; Magyar *et al.*, 1980a). Magyar *et al.* (1980a) showed that the fetal plasma cortisol concentrations increased significantly from day 11.8 ± 1.0 (SEM) before labour, and again during the last 3–4 days of uterine life, then decreased rapidly within the first week post partum. Cortisol concentrations in fetal plasma may, in fact, start to increase about 25 days before delivery (Norman *et al.*, 1985; Challis and Brooks, 1989).

Isotopic kinetic techniques showed that the cortisol present in the fetal circulation before 120 days of gestation is derived by transplacental transfer from the mother (Hennessy *et al.*, 1982), and it is only after this time that the fetal adrenal gland contributes. The increase in plasma cortisol concentrations may reflect an increase in the cortisol-binding capacity of fetal plasma (Fairclough and Liggins, 1975; Ballard *et al.*, 1982). Fetal infusion of ACTH at day 127 of gestation can stimulate an increase in the cortisol-binding capacity of fetal plasma (Challis *et al.*, 1984). The simultaneous administration to the fetus of metyrapone, an 11β-hydroxylase inhibitor, blocks the increase in cortisol binding capacity in the fetal circulation, suggesting that the increase in fetal plasma cortisol may be the stimulus for transcortin (CBG) production (Challis *et al.*, 1984).

10.3 ADRENOCORTICOTROPHIC HORMONE (ACTH)

The measurement of ACTH in ovine fetal plasma has been fraught with difficulties. In addition to $ACTH_{1-39}$ several large molecular weight precursors of ACTH are present in the fetal circulation (see Jones, 1983, for references). Most of the earlier radioimmunoassays (RIAs) for ACTH cross-reacted to some extent with these high molecular weight (HMW) forms and also small molecular weight species. This probably accounted for the higher resting values reported in earlier studies, i.e. 207 ± 151 pg/ml (Jones *et al.*, 1978a) and 366 ± 42 pg/ml (Rees *et al.*, 1975; Nathanielsz *et al.*, 1977). With the development of more specific antisera, the values measured were 69.6 ± 7.1 pg/ml (Wintour *et al.*, 1980), 20–30 pg/ml (Norman *et al.*, 1985) and 13.0 pg/ml (Pradier *et al.*, 1985). Less specific assays using antisera which cross-react with HMW ACTH peptides have considerable value if used in conjunction with chromatography to determine changes in the processing of the precursor hormone proopiomelanocortin (POMC) (Crosby *et al.*, 1988, 1989).

Using one of the less specific antisera, Jones and Roebuck (1980) and Jones (1983) showed that until 135–140 days of gestation, the HMW forms predominate in plasma, and that $ACTH_{1-39}$ represented only 25–30% of the total ACTH pool. From 140 days onwards the proportion of $ACTH_{1-39}$ relative to the HMW forms rose (Jones and Roebuck, 1980). The HMW forms of ACTH have a low steroidogenic potential (Gasson, 1979, 1980; Chatelain and Dupouy, 1980; Roebuck *et al.*, 1980; Durand *et al.*, 1984a; Brieu and Durand, 1987), and Jones (1983) argued that most previous studies on fetal plasma ACTH were misleading because the RIAs used measured both active and relatively inactive ACTH molecules. When measured after peptide separation by gel filtration and RIA, the concentration of $ACTH_{1-39}$ in fetal sheep plasma remained at low levels until day 135 before increasing markedly until term (Jones, 1983). Using preparative gel filtration of plasma extracts, preliminary data indicated that $ACTH_{1-39}$ increased in parallel with the major increase in plasma cortisol from day 140 onwards (Jones, 1983). However, measurements of 'total' immunoreactive ACTH concentrations failed to demonstrate significant changes before the prepartum increase in the fetal cortisol concentration (Rees *et al.*, 1975;

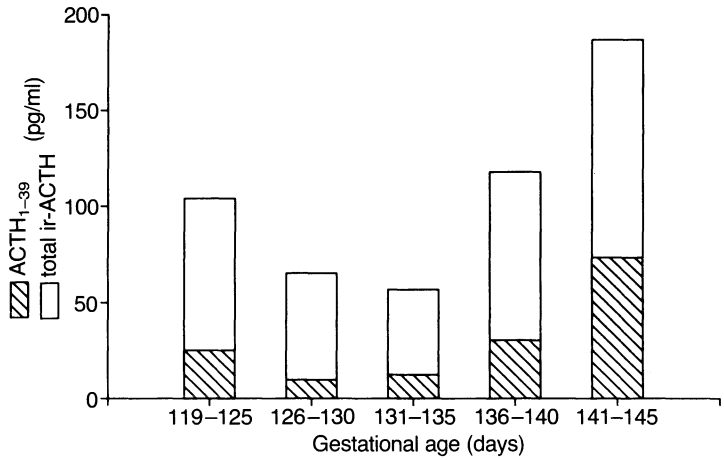

Figure 10.1 Fetal plasma concentrations of ACTH $_{1-39}$ and total immunoreactive ACTH in intact fetal lambs during late gestation. Samples were subjected to chromatography on Sephadex G50 fine and then quantitated by radioimmunoassay.

Jones *et al.*, 1977a; Wintour *et al.*, 1980; Rose *et al.*, 1982; Jones, 1983).

In contrast to the above findings, Norman *et al.* (1985), using a direct RIA which is reported to be more specific for $ACTH_{1-39}$, have shown a small progressive increase in fetal plasma ACTH during the last 30 days of pregnancy. The initial phase of the ACTH rise preceded or coincided with the increase in plasma cortisol, agreeing with the earlier data of Hennessy *et al.* (1982). Recent studies using an IRMA show a progressive increase in $ACTH_{1-39}$ concentrations during the last third of gestation (Deayton *et al.*, 1993)

Gel filtration chromatography (Sephadex G50) and subsequent RIA have indicated that $ACTH_{1-39}$ in fetal plasma increases over the last 20–25 days of gestation (Hollingworth *et al.*, 1990a), and that $ACTH_{1-39}$, calculated as a percentage of total immunoactivity, increases from ~15% at 126–130 days to ~40% at 141–145 days of gestation (Figure 10.1).

Recently, we have used a two-site IRMA (Crosby *et al.*, 1988) to measure directly the basal levels of the HMW ACTH precursor peptides (proopiomelanocortin [POMC, M_r 31K, K_{av} =0.08] and pro-ACTH [M_r22K]) and $ACTH_{1-39}$ as a function of gestational age. Under basal conditions, the concentration of the precursor peptides was high and did not change significantly during the last third of gestation (69±7 to 82.7±7 pmol/l at 110–114 and 140–142 days of gestation respectively; Carr *et al.*, 1992). However, basal $ACTH_{1-39}$ concentrations increased from 4.4±0.4 pmol/l (Carr *et al.*, 1992) at 110–114 days to 10.5±1.1 pmol/l at 140–142 days. The molar ratio of the HMW forms to $ACTH_{1-39}$ decreased from 15.7 at 110 days' to 7.9 at 140 days' gestation. These findings confirm and extend the earlier studies of Jones and Roebuck (1980) and Hollingworth *et al.* (1990a) and demonstrate that the bioactive $ACTH_{1-39}$ concentrations increase progressively during the last third of gestation while the concentration of the HMW species remains unchanged. The POMC and pro-ACTH peptides used in this IRMA were purified from peptides secreted by human small cell lung cancer cells (DMS–79; White *et al.*, 1987). POMC peptides in the culture medium were characterized by Sephadex G75 chromatography and specific immunoradiometric analysis.

The functions of the HMW ACTH peptides, which are present in high concen-

tration in fetal plasma, are still a matter of debate. It has been proposed that the HMW forms are weakly steroidogenic and possibly growth-promoting in low concentrations (Gasson, 1979; Chatelain and Dupony, 1980; Roebuck *et al.*, 1980; Durand *et al.*, 1984a). Some peptides derived from the N-terminal fraction of the POMC molecule also show biological activity in adrenal steroidogenesis (Pederson and Brownie, 1980; Al Dujaili *et al.*, 1981; Lis *et al.*, 1981). N-POMC-derived peptides (N-POMC-(1-61) amide), γ_2 melanocyte stimulating hormone (MSH) and γ_3-MSH potentiate the acute steroidogenic activity of $ACTH_{1-24}$ on ovine fetal adrenal cells (Durand *et al.*, 1984a). Other POMC-derived peptides, including β-LPH, also possess a trophic effect, these were shown to enhance the sensitivity of the fetal adrenal cells to the steroidogenic actions of $ACTH_{1-24}$ (Durand *et al.*, 1984a).

Fetal bilateral adrenalectomy markedly increased the proportion of $ACTH_{1-39}$ compared with intact controls, and $ACTH_{1-39}$ was present in substantial amounts (30% of total immunoactivity) from as early as 117 days of gestation.

In contrast, Jones and Roebuck (1980) have proposed that the HMW precursors of ACTH which are present in the fetal circulation may antagonize the stimulatory effect of ACTH on the adrenal earlier in gestation, and the preferential secretion of $ACTH_{1-39}$ late in gestation may explain the increase in fetal growth and cortisol secretion at that time, 1983). Durand *et al.* (1985a,b) stressed that, in the studies of Roebuck *et al.* (1980), a substantial reduction in ACTH-induced steroidogenesis was only obtained when the ratio of HMW forms of ACTH/$ACTH_{1-39}$ was about 10. This ratio was always <4 in the fetal pituitary (Silman *et al.*, 1979) and lower in fetal blood (Jones and Roebuck, 1980). However, it has recently been shown, using an IRMA, that the molar ratio of HMW species/$ACTH_{1-39}$ at days 110–114 clearly exceeded this figure, but by days 140–142, the ratio had decreased to 7.9 (Carr *et al.*, 1992). This decreasing ratio may explain the increase in fetal adrenal steroidogenesis as term approaches.

In contrast to earlier results, recent experiments provide strong evidence that POMC and pro-ACTH (used at concentrations found in fetal lamb plasma) inhibit $ACTH_{1-24}$ -induced secretion of cortisol from ovine fetal adrenal cells *in vitro* (Crosby *et al.*, 1993). By themselves, neither of the HMW peptides had any steroidogenic action on the adrenal cells. However, both independently inhibited $ACTH_{1-24}$-stimulated cortisol secretion from adrenal cells derived from fetal sheep between days 116 and 132 of gestation. In cells from fetuses greater than 140 days' gestation, pro-ACTH, but not POMC, consistently inhibited the action of $ACTH_{1-24}$, suggesting the effects of POMC vary as a function of gestational age. At physiological concentrations, these HMW peptides markedly lowered the maximum cortisol response of fetal adrenal cells to $ACTH_{1-24}$. These findings suggest that, at the concentrations of the HMW species found in fetal plasma (Carr *et al.*, 1992), the steroidogenic action of $ACTH_{1-39}$ (or $ACTH_{1-24}$) is severely blunted and that the fetal plasma cortisol concentrations required to induce parturition cannot be achieved until the concentrations of $ACTH_{1-39}$ present in the fetal circulation increase substantially. At the present time we lack detailed information on the plasma concentrations of the precursor peptides from day 140 until term. Clearly, this inhibitory action of the HMW species can be overridden by high concentrations of ACTH, since fetal infusion of $ACTH_{1-24}$ will initiate premature labour. In the days preceding parturition, the plasma $ACTH_{1-39}$ concentrations may have increased sufficiently to override the inhibitory action of the HMW species.

It has been argued that, after fetal hypophysectomy, the fetal adrenal gland should be more sensitive to ACTH due to the removal of the large molecular weight 'inhibi-

tory' peptides. Some peptides derived from the N-terminal fraction of the POMC molecule show biological activity in adrenal steroidogenesis (Pedersen and Brownie, 1980; Al Dujaili *et al.*, 1981; Lis *et al.*, 1981). N-POMC-derived peptides ($N\text{-}POMC_{1-61}$) amide, γ_2-melanocyte-stimulating hormone (MSH) and γ_3-MSH potentiate the acute steroidogenic activity of $ACTH_{1-24}$ on ovine fetal adrenal cells (Durand *et al.*, 1984a). Other POMC-derived peptides, including β-LPH, also possess a trophic effect and enhance the sensitivity of the fetal adrenal cells to the steroidogenic actions of $ACTH_{1-24}$ (Durand *et al.*, 1984a). It might be expected that the adrenal glands would be less responsive to $ACTH_{1-24}$ after hypophysectomy, which removes all these other peptides, and when $ACTH_{1-24}$ was infused into fetuses hypophysectomized 7–16 days earlier the fetal cortisol concentrations were substantially lower than those in intact fetuses (Kendall *et al.*, 1978b). These results suggest that, without the pituitary, the fetal adrenal is less responsive to ACTH and that HMW species may exert an important trophic action on the fetal adrenal and enhance its responsiveness to $ACTH_{1-24}$ or $ACTH_{1-39}$. Removal of pituitary hormones *in vivo* for 6 days (by fetal hypophysectomy) resulted in a decreased ability of cultured adrenal cells from these fetuses to produce cAMP and corticosteroids in response to $ACTH_{1-24}$ compared with cells from control fetuses (Durand *et al.*, 1985a), although Connors and Liggins (1980) saw no change in the responsiveness of fetal adrenals to ACTH within 24 h after hypophysectomy. The secretion of large molecular weight POMC species by the fetal pituitary may therefore: (i) assume a trophic role for the fetal adrenal, preparing it for the increased levels of $ACTH_{1-39}$ which will follow, and (ii) modulate the action of $ACTH_{1-39}$ released at the same time under stressful conditions and thus exert a protective role against the premature release of ACTH.

There may also be extrapituitary factors which inhibit fetal adrenal activity (Durand *et al.*, 1982a, 1985a,b). PGE_2 produced by the placenta may exert an important trophic action on the HPA (section 10.10.1). Recent studies have shown that, following fetal hypophysectomy at 125 days' gestation, a continuous infusion of very small amounts of $ACTH_{1-24}$ (115 pmol/h/kg) starting immediately after surgery, induced delivery at term (Jacobs *et al.*, 1993). Using an IRMA, the concentration of $ACTH_{1-24}$ in carotid artery plasma of hypophysectomized fetuses during the infusion was undetectable (less than 2.2 pg/ml) throughout the study, whereas the fetal plasma concentration of $ACTH_{1-39}$ in age-matched control fetuses ranged from a mean of approximately 35 pg/ml at day 135 to 160 pg/ml at term (day 147). In this study, only very low concentrations of bioactive ACTH were required to induce normal adrenal growth, a normal preparturient increase in fetal plasma cortisol concentrations and delivery at term. The authors (Jacobs *et al.*, 1993) concluded that in the absence of the pituitary, the fetal adrenal glands become more responsive to ACTH, presumably due to the removal of pituitary-derived inhibitory factors such as POMC and pro-ACTH. This study also showed that, in this preparation, the action of ACTH is permissive in that no increase in plasma ACTH concentrations was needed and, given very small amounts of ACTH, the adrenal gland itself can initiate parturition at normal term. Presumably, in normal pregnancies, the adrenal 'clock' is overridden by a central (hypothalamic?) 'clock' which determines the timing of the increase in bioactive $ACTH_{1-39}$ against an inhibitory background of HMW ACTH peptides. Clearly, this study focuses attention on the importance of the fetal adrenal glands in the timing of parturition. Another novel feature of these studies (Crosby *et al.*, 1993; Jacobs *et al.*, 1993) is they suggest that different products ($ACTH_{1-39}$ and POMC, pro-ACTH) of the same pro-hormone may act as

agonists and antagonists of their target organ, the fetal adrenal gland.

10.4 DEVELOPMENT OF FETAL ADRENAL FUNCTION

The increase in fetal plasma cortisol concentrations, starting about 10–12 days before parturition (Magyar *et al.*, 1980a), coincides with an enhanced responsiveness of the fetal adrenal to $ACTH_{1-24}$ (Liggins *et al.*, 1977). Before that time the immature fetal adrenal cortex responds poorly to short (1–2 h) infusions of $ACTH_{1-24}$ (10 μg/h) (Bassett and Thorburn, 1973; Liggins *et al.*, 1977; Brown *et al.*, 1978), yet a similar infusion into the mother caused a very rapid increase in maternal corticosteroid concentrations. The plasma cortisol response to $ACTH_{1-24}$ indicated that fetal adrenal sensitivity increased as a function of age late in gestation (Rose *et al.*, 1982). Although hypoxaemia raised the ACTH concentration in fetal plasma, the increase in ACTH was associated with a large and rapid increase in plasma corticosteroid concentrations only after day 139 (Jones *et al.*, 1977b). Similarly, the increase in plasma ACTH concentrations in response to haemorrhage (Rose *et al.*, 1978) and hypotension (Rose *et al.*, 1981) occurs without any significant corticosteroid response until late in gestation (0.89–0.98 of gestation). Although the bioactivity of the ACTH peptides released in these experiments is still uncertain (section 10.3), the pattern of responses of the fetal adrenal gland, as judged by the cortisol concentrations, was similar to that observed with $ACTH_{1-24}$, indicating that an increase in adrenal sensitivity late in gestation (during the last 10–12 days) corresponded to the increase in fetal plasma cortisol concentrations (Magyar *et al.*, 1980a). The increase in cortisol concentrations is an expression of the release of bioactive $ACTH_{1-39}$ from the fetal anterior pituitary gland.

The adrenal response to corticotrophin-releasing factor (CRF) occurs by 110–120 days' gestation, with a marked response at 125 days' gestation (Norman *et al.*, 1985; Pradier *et al.*, 1985). Norman *et al.* (1985) observed a significant increase in the plasma cortisol concentrations in normal fetuses as early as 125–130 days' gestation, i.e. some 25 days before delivery. The gestation length of sheep with chronically instrumented fetuses is often several days shorter than the mean gestation length of the flock and can vary between laboratories, sheep breeds and the number of experiments carried out on the fetus. Cortisol assays also vary between laboratories and it is easier to detect small changes in cortisol concentrations with more sensitive and specific assays. Nevertheless, the cortisol response to CRF in the above studies occurred earlier and was more marked than expected and raises the possibility that the pituitary (and or placental) factors released by CRF are more steroidogenic than $ACTH_{1-24}$. Another index of increased trophic drive to the fetal adrenal gland is its change in weight: the fetal adrenal gland grows in parallel to the growth of the fetus until day 130–135 when there is a rapid growth surge (Comline and Silver, 1961; Liggins, 1969a), indicating that significant amounts of $ACTH_{1-39}$ do not appear in the fetal circulation until this time.

10.5 FETAL MATURATION – *IN VITRO* STUDIES

In a perifusion system, the corticosteroid production of adrenal tissue from fetal lambs (100–145 days' gestation) in response to $ACTH_{1-24}$ increased gradually until 137 days and then increased markedly until term (145 days) (Bassett and Thorburn, 1973; Madill and Bassett, 1973). This increase paralleled the increases in adrenal weight and plasma corticosteroid concentration which occurred before birth (Madill and Bassett, 1973). It was suggested that *in vivo* the adrenal may well be responding at near-maximal rates to stimulation by endogenously produced ACTH dur-

ing the last 7–10 days of gestation, although this is clearly not so after birth (Madill and Bassett, 1973). These studies showed that, although the adrenals of younger fetuses can respond acutely to ACTH stimulation, it is the *in vivo* maturation of the ACTH-responsive system of the fetal adrenal which is rate limiting in the marked increase in corticosteroid concentrations during the last week before parturition. The studies of Madill and Bassett (1973) illustrate that the fetal adrenal depends on ACTH to maintain its steroidogenic capacity. The weight of the adrenals, the plasma cortisol concentration and the response of the adrenals to ACTH increase in parallel.

When $ACTH_{1-24}$ is infused over 3–4 days in amounts that result in supraphysiological concentrations in the plasma, fetal adrenal maturation is induced, but it is not known whether ACTH in physiological concentrations is responsible by itself for the normal preparturient increase in fetal adrenal responsiveness. An extrapituitary inhibitor of adrenal maturation (Durand *et al.*, 1982a, 1985a,b) and PGE_2, released from the placenta (section 10.10.1), has been suggested to play a role in fetal adrenal maturation.

Although the acute response of the fetal adrenal to ACTH is relatively weak before about 136–140 days' gestation, many studies have shown that prolonged infusion of $ACTH_{1-24}$ (4–10 μg/h) before that time can induce premature adrenal maturation, with high levels of cortisol in the fetal plasma at delivery (Liggins, 1968; Bassett and Thorburn, 1973; Strott *et al.*, 1974; Kendall *et al.*, 1977). The doses used are probably pharmacological and exceed the calculated production rate of $ACTH_{1-39}$ (40 ng/h) by a factor of 50–200 (Jones *et al.*, 1975; Nathanielsz *et al.*, 1977). When the dose of $ACTH_{1-24}$ was reduced to 0.25 μg/h, starting at day 129, two fetuses were delivered at 143 and 144 days' gestation, suggesting possible premature delivery (Cabalum *et al.*, 1982). Clearly this dose of $ACTH_{1-24}$ must have been close to or at the threshold: it would be of value to measure the plasma concentrations of ACTH and cortisol in hypophysectomized and control fetuses infused with 100–200 ng/h of $ACTH_{1-24}$ from 120 days' gestation. This amount of ACTH should provide sufficient trophic support for the fetal adrenal. If the hypophysectomized fetuses (with ACTH infusion) are delivered at normal term, it would suggest that the trigger for parturition may reside in the adrenal or the placental–adrenal axis.

Recently, these questions have been addressed experimentally. Fetuses hypophysectomized at 125 days' gestation and infused with 0.2 μg/h $ACTH_{1-24}$ delivered prematurely while those infused with 0.1 μg/h delivered at term (Jacobs *et al.*, 1993). These experiments demonstrate how exquisitely sensitive the fetal adrenal glands are to biologically-active ACTH in the absence of the pituitary.

Because of their earlier observations and those of others indicating that many peptide hormones are secreted in a pulsatile fashion, Lye *et al.* (1983) compared the effect of administering ACTH as pulses (66 ng/min given for 15 min every 2 h) with giving the same dose (990 ng per 2 h) as a continuous infusion. This dose of ACTH (~500 ng/h) is half the dose (1 μg/h) used by Cabalum *et al.* (1982) but only twice the apparent threshold (see above). Pulsatile administration of ACTH was continued until premature delivery occurred with a mean time interval of 99.1 h for singleton fetuses. Both the continuous and pulsatile modes of administration of $ACTH_{1-24}$ provoked a significant increase in the fetal plasma cortisol concentrations (Lye *et al.*, 1983). Unfortunately, plasma concentrations of ACTH were not measured. However, increasing the $ACTH_{1-24}$ dose from 250 ng/h (Cabalum *et al.*, 1982) to 500 ng/h (Lye *et al.*, 1983) was sufficient to induce premature parturition, suggesting that the system is very sensitive to a small change in ACTH concentrations.

10.6 BIOCHEMICAL CHANGES IN THE FETAL ADRENAL ASSOCIATED WITH MATURATION

The fetal adrenal responds *in vitro* to ACTH with maximum cortisol output per unit weight of gland early in pregnancy (days 50–60); the capacity is reduced during mid-pregnancy but returns again near term (Wintour *et al.*, 1975). It has been suggested that there is a suppression or inhibition of adrenal function around day 100 (mid-gestation) (Durand *et al.*, 1982a, 1985a,b) and the withdrawal of this inhibition may be responsible for the increased adrenal responsiveness to ACTH (adrenal maturation) that precedes parturition. A lack of circulating $ACTH_{1-39}$ (or the presence of HMW species) would also explain this so-called inhibition because the infusion of $ACTH_{1-24}$ can reverse it.

Administration of $ACTH_{1-24}$ to fetuses after day 100 of gestation causes premature maturation of fetal adrenal function (Challis *et al.*, 1982a; Durand *et al.*, 1982b, 1984b; Lye *et al.*, 1983; Cathiard *et al.*, 1985, Challis and Brooks, 1989). Specifically, the changes in the adenylate cyclase system and steroidogenic enzymes resemble those seen before normal-term parturition. Infusion of $ACTH_{1-24}$ (100 μg/day for 5 days) into 115-day ovine fetuses caused a marked increase in the ability of adrenal cells to produce both cAMP and corticosteroids, when stimulated by $ACTH_{1-24}$ or cholera toxin (Durand *et al.*, 1981a; Challis *et al.*, 1982b). The changes in the cell membrane included:

1. an increased activity of the regulatory and/or catalytic subunits of adenylate cyclase as demonstrated by a greater response of cyclase to both sodium fluoride and guanylylimidodiphosphate [Gpp(NH)p] compared with that of control fetuses;
2. the appearance of a more efficient 'coupling' between ACTH receptors and adenylate cyclase, because $ACTH_{1-24}$, which is unable to increase cyclase activity in membranes from control fetuses, stimulated this enzyme in membranes from $ACTH_{1-24}$-treated fetuses;
3. an increased number of ACTH receptors from 47 ± 4 to 106 pmol per μg of DNA (Durand *et al.*, 1981b).

10.7 ACTH RECEPTORS IN THE FETAL ADRENAL

Recent studies have been directed towards elucidating changes in the number of receptors for ACTH (Durand, 1979), in the activity of adrenal adenylate cyclase (Durand *et al.*, 1981a–c) and in activities of the enzymes operant in the cortisol biosynthetic pathway (Anderson *et al.*, 1970, 1972; Madill and Bassett, 1973). ACTH binding sites have been identified in cell membrane preparations of fetal sheep adrenal glands. Determination of the affinity of the binding sites by Scatchard analysis gives an apparent K_d of about 10^{-7} M, a lower binding affinity than normally associated with membrane receptors (Durand, 1979). However, the K_d of the receptor–hormone binding and the apparent ED_{50} of adenylate cyclase activation by ACTH ($2–5 \times 10^{-8}$ M) are reported to be well correlated (Durand *et al.*, 1985b). There is another small pool of high-affinity sites in adult adrenal membranes (K_d 10^{-12} M or 10^{-10} M) (Lefkowitz *et al.*, 1971; McIlhinney and Schulster, 1975). The hypothesis of a dual messenger function in ACTH action on adrenal steroidogenesis has received considerable attention (Li *et al.*, 1989; Gallo-Payet and Payet, 1989). ACTH is now known to stimulate steroidogenesis by at least two mechanisms. Both appear to involve specific cell membrane receptors, but only one class of receptor recruits the formation of cAMP in the signal transduction process. It has also been proposed that mobilization of intracellular calcium mediates steroidogenesis in response to a receptor species independent of adenylate cyclase (Yanagibashi, 1979; Kojima *et al.*, 1985; Rasmussen *et al.*, 1986). More-

over, low concentrations of ACTH can stimulate steroid secretion without detectable changes in intracellular cAMP, and can induce calcium influx as well as a rise in cytosolic calcium (for references see Gallo-Payet and Payet, 1989). Accordingly, Gallo-Payet and Payet (1989) have postulated that, at low concentration, ACTH binds preferentially to the high-affinity site of its receptor (type I), leading to calcium influx by depolarization of the membrane potential, and to steroid secretion predominantly through an inositol phosphate (IP_3)- and Ca^{2+}-stimulated pathway. At higher concentrations, ACTH also binds to a lower affinity site of its receptor (type II), largely stimulating cAMP production and further increasing steroid secretion. Li *et al.* (1989) have proposed that $ACTH_{1-10}$ and $ACTH_{11-24}$ promote adrenal steroidogenesis by different mechanisms. Using bovine adrenal cells they found that one class of receptor recognized $ACTH_{1-10}$, but not $ACTH_{11-24}$, and that it was linked to the cAMP messenger pathway. In contrast, $ACTH_{11-24}$ was linked to the IP_3 pathway. Presumably $ACTH_{1-24}$ works through both the type I (IP_3/Ca^{2+}) and type II (adenylate cyclase) pathways. It is therefore of considerable interest that $ACTH_{11-24}$ displaces ^{125}I-labelled $ACTH_{1-24}$ from its binding sites on ovine fetal adrenal preparations, whereas $ACTH_{1-10}$ does not (Durand *et al.*, 1985b). This would suggest that the type I receptor and its associated IP_3/Ca^{2+} pathway is present in fetal membranes (between 100 and 130 days' gestation), whereas the type II receptor and its associated cAMP pathway is not.

The number of $ACTH_{1-24}$ binding sites, expressed per μg of DNA, remains constant from day 124 to day 140, then increases dramatically (threefold) between day 140 and term (Durand *et al.*, 1985b). The close temporal relationship between the increase in adrenal adenylate cyclase responsiveness, the plasma corticosteroid concentrations and the number of adrenal ACTH receptors from day 140 supports the functional specificity of these binding sites and strongly suggests that the increase in their number plays an important role in the preparturient maturation of the fetal adrenal (Manchester and Challis, 1982; Durand *et al.*, 1985b). It also indicates that the preparturient increase in ACTH receptors is closely linked to adenylate cyclase activity of the receptors and suggests that the receptors are of type II. Adrenal cells between 100 and 130 days' gestation have high levels of ACTH receptors, but their response to ACTH, as judged by adenylate cyclase activity, is poor (Durand *et al.*, 1981a–c). At 124 days' gestation, $ACTH_{1-24}$ was not able to increase adenylate cyclase activity in crude membrane preparations from ovine fetuses. It is possible, therefore, that the ACTH receptors present in the fetal adrenal between 100 and 130 days are type I and are associated with the IP_3/Ca^{2+} pathway, and that the increase in receptors during the last 7–10 days is due to a marked increase in type II receptors which account for the increase in adenylate cyclase activity and steroidogenesis. The loss of sensitivity of the fetal adrenal in mid-gestation may be associated with the loss of type II receptors (due to low $ACTH_{1-39}$ concentrations), and the biochemical changes observed at this time may be equivalent to a functional hypophysectomy with a selective loss of type II receptors. This situation can be reversed by the infusion of $ACTH_{1-24}$ into young fetuses, in which the changes in cAMP and corticosteroid production closely resemble those that occur spontaneously at term (Durand *et al.*, 1981b) and with $ACTH_{1-24}$ infusion of hypophysectomized fetuses (Jones *et al.*, 1978b). It has been shown in adult hypophysectomized rabbits that ACTH alone can induce its own (presumably type II) receptors, and so the preparturient increase in ACTH receptors may be due to a direct effect of ACTH.

It is therefore possible that, with very low levels of ACTH in mid-gestation, the numbers of type II receptors are lost and post-receptor changes occur. At day 63, ACTH,

cholera toxin, Gpp(NH)p and forskolin all stimulated cAMP and cortisol output by ovine fetal adrenal cells (Manchester and Challis, 1982; Durand *et al.*, 1985b, 1987; Challis and Brooks, 1989). Responses to these agonists were lost at day 100 but reappeared near term (Durand *et al.*, 1981a; Manchester and Challis, 1982). Durand *et al.* (1981b) were unable to stimulate adenylate cyclase activity with ACTH using adrenal membranes from day 124 fetuses, although sodium fluoride and Gpp(NH)p were effective. By day 140, however, the ACTH effect had reappeared and was enhanced by Gpp(NH)p. Manchester and Challis (1982) concluded that their inability to stimulate steroid output in response to ACTH between days 100 and 130 could relate to the presence of a population of ACTH receptors that are not functionally linked to adenylate cyclase. However, the receptors may be linked to the IP/Ca^{2+} pathway. Although in these studies there was no steroidogenic response to ACTH, earlier *in vitro* studies (Glickman and Challis, 1980; Magyar *et al.*, 1980b) had shown a small response to ACTH, and Norman *et al.* (1985) and Pradier *et al.* (1985) found that CRF administration to fetuses between 115 and 130 days' gestation caused a small but significant increase in cortisol concentrations; this response may be mediated by the type I receptor.

It has been suggested that the block to adrenal responsiveness after day 124 is due to decreases in GTP availability or GTP binding (Durand *et al.*, 1981b), and that coupling through the G_s protein is probably a rate-limiting step in cAMP production (Durand *et al.*, 1985b). It assumed that appropriate (possibly type II) receptors are present before 140 days but the available data would be consistent with a lack of adenylate cyclase-coupled (type II) receptors. These receptors can be induced *in vivo* or *in vitro* by ACTH treatment. As the studies of Li *et al.* (1989) indicate that ACTH acts via both receptors simultaneously, the results with $ACTH_{1-24}$ suggest that, if only the type I receptor is present (e.g. between 100 and 130 days' gestation), the steroidogenic response, although present, would be greatly reduced. The appearance of the type II receptor (e.g. at 137–140 days) would greatly enhance the steroidogenic response to ACTH. If the development of adenylate cyclase activity in the fetal adrenal membranes is dependent on the presence of biologically active ACTH in the fetal circulation, it would suggest that $ACTH_{1-39}$ concentrations in fetal plasma must be low until about 137 days' gestation or inadequate to induce the adenylate-coupled (type II) receptors.

In summary, the increase in fetal cortisol concentrations occurs in two phases the first, starting 20–25 days before delivery, is small and gradual and the second, starting some 4–5 days before delivery, is rapid and marked. It is possible that $ACTH_{1-39}$ at low levels (or the HMW species of POMC at high concentrations) acts via the high-affinity type I receptors to produce low levels of steroidogenesis and low cortisol concentrations, and that with increasing $ACTH_{1-39}$ concentrations the type II receptors are induced and steroidogenesis and cortisol secretion increase markedly.

10.8 INHIBITION OF FETAL ADRENAL FUNCTION

Adrenal cells from 115 to 120-day ovine fetuses, when cultured for 6 days, underwent spontaneous maturation of their response to $ACTH_{1-24}$ (Durand *et al.*, 1981a, 1982a, 1985a,b). In cells maintained in ACTH-free media, basal cAMP production remained low and constant; an exponential increase in their cAMP response to a 2-h treatment of $ACTH_{1-24}$ was observed, and by day 6 the response was 25-fold higher than on day 1. When cells were exposed to $ACTH_{1-24}$ for 2 h per day from day 1 onwards, the development of the cAMP response was faster, but

the production of cAMP was only slightly greater than that of cells cultured in ACTH-free media (Durand *et al.*, 1982a). Durand *et al.* (1982a) therefore postulated the existence in the ovine fetus of factor(s) which inhibit the 'spontaneous' development *in vivo* of some component(s) of the $ACTH_{1-24}$-sensitive adenylate cyclase system. The action of the putative inhibitory factor(s) can be overcome by infusing fetal lambs with $ACTH_{1-24}$. In some cell systems, calcium can antagonize the actions of cAMP: calcium–calmodulin complex activates cAMP phosphodiesterase, the enzyme that catalyses the metabolism of cAMP to the inactive 5'-AMP. An increase in free cytosolic calcium ion can therefore decrease the levels of cAMP and cAMP-dependent protein kinase activity and change the activities of the enzymes that are substrates for this kinase (Mendelson, 1988). It is possible that an inhibitory factor might work via such a system and suppress cAMP levels. Oka *et al.* (1989) have shown that angiotensin II and transforming growth factor beta (TGF-β), both of which are known to act through IP_3/Ca^{2+} receptors, can block the stimulatory actions of ACTH on cortisol production in cultured bovine fetal adrenal cells. In ovine fetal adrenal cells angiotensin II and TGF-β inhibited the ACTH induction of cortisol production and $P450_{17\alpha}$ expression (Rainey *et al.*, 1991). The putative inhibitor(s) could be therefore acting via this IP_3/Ca^{2+} pathway. However, at low concentrations, ACTH binds to high-affinity sites (section 10.7), leading to calcium influx through voltage-dependent Ca^{2+} channels secondary to decreased potassium permeability, leading to steroid secretion by the IP_3/Ca^{2+} pathway (see Gallo-Payet and Payet, 1989). This receptor pathway may operate in the fetal adrenal despite the functional absence of the adenylate cyclase pathway. It is possible that at low concentrations, $ACTH_{1-39}$ binds to the high-affinity sites, producing low levels of cortisol and inhibiting the adenylate cyclase pathway. However, as $ACTH_{1-39}$ concentrations increase, adenylate receptors increase, thereby stimulating steroidogenesis.

Challis *et al.* (1984) have shown that trifluoperazine, a calmodulin antagonist, blocks both ACTH- and dibutyryl cAMP-stimulated cortisol output by term fetal adrenal cells, providing further evidence that the calmodulin system is involved in post-receptor events in the fetal adrenal. In addition, the calcium channel blockers nifedipine and verapamil inhibit steroidogenesis in bovine adrenal cells induced by $ACTH_{1-10}$, $ACTH_{11-24}$ or $ACTH_{1-24}$ (Li *et al.*, 1989), suggesting that calcium is required for release of steroid from adrenal cells regardless of the receptor/effector mechanism.

10.9 STEROIDOGENIC PATHWAYS

Madill and Bassett (1973) showed that cortisol (F) was the principal corticosteroid released by the fetal adrenal at term, the molar ratio of F to corticosterone (B) in the perfusion eluate increased from 1.2 for tissues from fetuses of 100–200 days' gestation, to 2.5 at 135 days. In older fetuses, the ratio varied between 1.5 and 12.5 with a mean of 5.3 (± 1.1) compared with 7.1 ± 0.3 in lambs and 10.5 ± 2.3 in adult sheep. Alexander *et al.* (1968) found a similar increase in the F/B ratio in adrenal venous plasma as the fetus neared term. Madill and Bassett (1973) considered the changes in the F/B ratio reflected an activation of 17α-hydroxylase by ACTH. Wintour *et al.* (1977) reported the inability of adrenal quarters from fetuses of 90–120 days' gestation to produce F in response to ACTH. Anderson *et al.* (1970) suggested that both 17α and 11β hydroxylating enzyme systems were relatively inactive in the adrenal cortex of the fetal lamb at 122 days' gestation and that these activities increased greatly just before term or after prolonged fetal infusion of ACTH (Anderson *et al.*, 1972).

In an attempt to elucidate the change in activities of enzymes involved in corticosteroid synthesis, Manchester and Challis (1982)

measured the formation of F and B in the presence of pregnenolone (P_5), 17α-hydroxypregnenolone (17α-OHP_5), progesterone (P_4) and 17α-hydroxyprogesterone (17α-OHP_4). In Day 50 tissue all four substrates were converted to F, and P_5 and P_4 were converted to B, showing the presence of 11β- and 21-hydroxylase activities as well as 5,3β-hydroxysteroid dehydrogenase. By Day 100 only 17α-hydroxysteroids were converted to F and the conversion of P_4 to 17α-OHP_4 was low. By day 130 and at term, both P_4 and 17α-OHP_4 were converted to F. It was concluded that there was a marked increase in 17α-hydroxylase activity during the later part of gestation and at term it was no longer rate limiting for cortisol production. The activity of 3β-hydroxysteroid dehydrogenase enzyme also increased during late gestation, but at term it remained, relatively, a rate-limiting enzyme (Manchester and Challis, 1982). Similar conclusions were reached by Durand *et al.* (1982b).

10.10 PROSTAGLANDIN E_2

Placental prostaglandin (PG) synthesis, as judged by the PG synthetic capacity of cotyledonary microsomes (Rice *et al.*, 1988) and isolated placental cells (Risbridger *et al.*, 1985), increases markedly from day 100 onwards. The ovine placenta secretes increasing amounts of PGE_2 into the fetal and maternal circulations (Fowden *et al.*, 1987), in parallel with the increase in fetal cortisol (Thorburn *et al.*, 1988; Thorburn and Rice, 1990). The increase in PGE_2 precedes that of ACTH or cortisol (S.A. Hollingworth, personal communication) and the placenta, by releasing PGE_2, may play an important role in the activation of the fetal HPA axis (Rice and Thorburn, 1988; Thorburn *et al.*, 1988, 1989, 1991; Thorburn and Rice, 1990).

10.10.1 CENTRAL ACTIONS OF PGE_2

The PGs have been implicated in the regulation of the activity of the HPA axis. PGE_2 stimulates ACTH release from the pituitary, and PGs of the E series may act at a suprapituitary site because their action is blocked by the administration of morphine or lesions in the median eminence (de Wied *et al.*, 1969; Peng *et al.*, 1970). Local administration of PGs of the E, F, A and B series stimulate ACTH secretion when injected into medial basal hypothalamus at doses (0.5–1 μg) that are without effect when injected into the lateral basal hypothalamus, the anterior pituitary or the tail vein (Hedge, 1977, for review). Local hypothalamic administration of indomethacin completely prevents compensatory adrenal hypertrophy as well as the ACTH secretion induced by some stresses (Thompson and Hedge, 1978), suggesting that local synthesis of PGs in the hypothalamus can influence the secretion of corticotrophins.

The infusion of PGE_2 into the abdominal aorta (Vilhardt and Hedqvist, 1970) or of PGE_1 intravenously (Berl and Schrier, 1973) causes the release of vasopressin (AVP) and is probably, at least in part, a centrally mediated effect (Vilhardt and Hedqvist, 1970; Andersson and Leksell, 1975; Yamamoto *et al.*, 1976). Further evidence of a direct action of PGE_2 on the hypothalamus was the finding that PGE_2 was also active in an explanted guinea pig hypothalamo-neuro hypophysial complex (Ishikawa *et al.*, 1981). Since PGs are formed in the hypothalamus (Ojeda *et al.*, 1978; Hayaishi, 1983), it has been proposed that PGs exert a stimulatory action on ACTH release by increasing secretion of the ACTH-releasing hormones, CRF and AVP (Hedge, 1977; Thompson and Hedge, 1978). It is possible that PGs produced in the hypothalamus may be carried in the portal circulation to act on the pituitary.

PGE_2 stimulates the hypothalamus to release ACTH but its action on the pituitary is thought to be inhibitory. Several *in vitro* studies have shown that PGE_2, but not $PGF_{2\alpha}$, PGD_2 or PGI_2, inhibits the release of ACTH/β-endorphin after stimulation of the anterior pituitary by AVP or CRF_{1-41} (Vale *et*

al., 1978; Vlaskovska *et al.*, 1984; Vlaskovska and Knepel, 1984). Specific PGE_2 binding sites exist in the anterior pituitary gland (Malet *et al.*, 1982) and PGE_2 is the major PGH synthase (cyclooxygenase) product produced by the gland (Ojeda *et al.*, 1978; Pilote *et al.*, 1982; Vlaskovska *et al.*, 1984). AVP and CRF stimulated the secretion of PGE_2 by the anterior pituitary *in vitro* (Vlaskovska *et al.*, 1984). Thus it has been proposed that PGE_2 is formed locally in the anterior pituitary following CRF and/or AVP binding to the gland and may act as a negative-feedback modulator of ACTH/β-endorphin release and that the effects of blockade of the PGH synthase may be secondary to the removal of a local negative-feedback signal (PGE_2) (Vlaskovska and Knepel, 1984).

When indomethacin or other PGH synthase inhibitors are administered systemically (Weidenfeld *et al.*, 1980, 1981; Thompson and Hedge, 1981) or directly into the hypothalamus (Thompson and Hedge, 1978) ACTH secretion is stimulated. The major site of action of systemically injected indomethacin is considered to be the adenohypophysis and the stimulation is probably secondary to the removal of the local inhibitory control of PGE_2 on pituitary ACTH release.

Vlaskovska and Knepel (1984) have proposed that lipoxygenase-derived arachidonic acid metabolites may exert a stimulatory action on ACTH release from the pituitary. In this study the major metabolite of arachidonic acid in the adenohypophysis was found to be 12-OH-5,8,10,14-eicosatetranoic acid (12-HETE), indicating the relative importance of the C_{-12} lipoxygenase pathway of arachidonic acid metabolism (Pilote *et al.*, 1982). Nordihydroguaiaretic acid (NDGA), which inhibits lipoxygenase activity in the adenohypophysis and reduces the formation of 12-HETE, inhibited the release of β-endorphin in response to stimulation by AVP or CRF, although 12-HETE had no effect on ACTH release (Vlaskovska and Knepel, 1984). The inhibitory action of PGE_2 may also be mediated by the lipoxygenase pathway since PGE_2 no longer inhibited AVP-induced β-endorphin release in the presence of NDGA (Vlaskovska and Knepel, 1984). The potent stimulatory effect of indomethacin on ACTH release may therefore be due to a block of PGH synthase activity, thereby removing the inhibitory action of PGE_2 on the lipoxygenase pathway, and by the preferential shunting of arachidonic acid into the lipoxygenase pathway with the formation of stimulatory metabolites. The administration of PGH synthase inhibitors [4-amino antipyrine (4-AAP) and indomethacin] dramatically increases ACTH concentrations in adrenalectomized fetuses, suggesting that similar mechanisms may be operative in the fetal lamb (Hollingworth *et al.*, 1990b). When PGE_2 (2 μg/min) is infused into fetal lambs from day 110 onwards, there is a pronounced increase in plasma ACTH values (Hollingworth *et al.*, 1990c; Thorburn, 1991; Thorburn *et al.*, 1991). Chromatographic analysis of the plasma on Sephadex G50 columns revealed that PGE_2 stimulated the release of high and low molecular weight precursors as well as $ACTH_{1-39}$ (Figure 10.2).

PGE_2 appears to act on ACTH release at the level of the hypothalamus, because ACTH release does not occur in response to PGE_2 in hypothalamic–pituitary disconnected (HPD) fetuses. When the PGE_2 infusion into the fetus was prolonged for ⩾24 h, fetal plasma ACTH values declined to basal levels after 12 h, although cortisol concentrations remained elevated during the infusion (Thorburn *et al.*, 1991). The changes in ACTH and cortisol concentrations were similar to those during prolonged fetal hypoxaemia caused by a restriction in placental blood flow (Challis *et al.*, 1989; Hooper *et al.*, 1990). The decrease in ACTH values probably resulted from the inhibitory feedback of cortisol, and the prolonged increase in cortisol was probably due to a direct action of PGE_2 on the fetal adrenal (Louis *et al.*, 1976).

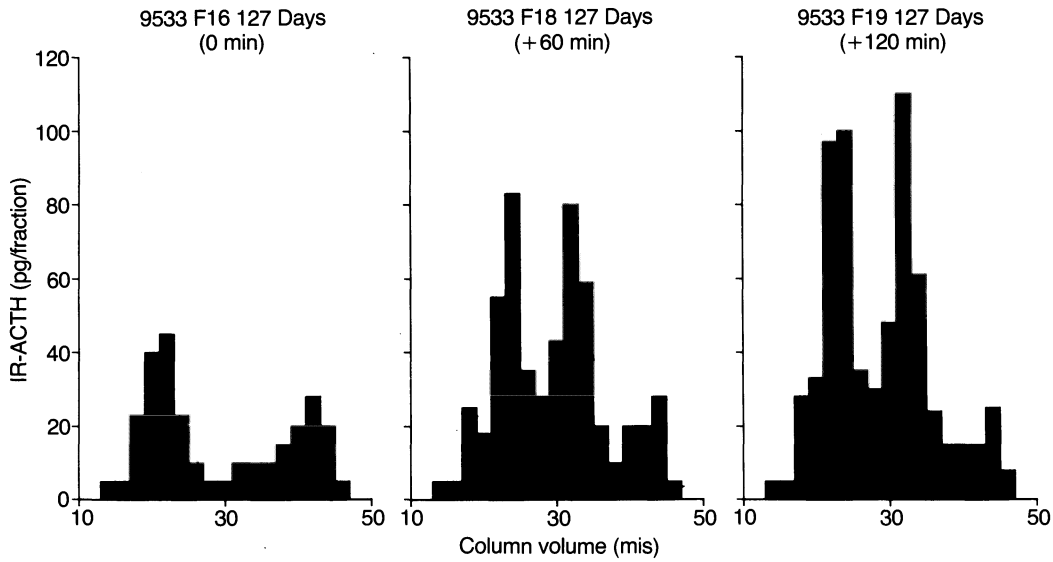

Figure 10.2 Elution profiles of immunoreactive (IR) ACTH in fetal plasma. Samples were collected at 0, 60 and 120 min after the infusion of prostaglandin E_2 (2 μg/min) into an intact fetus at 127 days' gestation. $ACTH_{1-39}$ elutes at 31 ml. Samples (1.0 ml) were subjected to gel filtration chromatography on Sephadex G50 fine using 1% formic acid (containing Polypep) as elution buffer at a flow rate of 10 ml/h.

10.10.2 PGE_2 ACTION ON THE FETAL ADRENAL

In adult animals exogenous PGEs stimulate adrenocortical cAMP production (Sarutz and Kaplan 1972; Honn and Chavin, 1977) and increase adrenal steroid production and release (Flack and Ramwell, 1972; Sarutz and Kaplan, 1972; Laychock and Rubin, 1975; Warner and Rubin, 1975; Ellis *et al.*, 1978). Prostacyclin is more potent than PGE_2 in stimulating steroidogenesis (Ellis *et al.*, 1978; Liggins *et al.*, 1982). The presence of PGs, particularly PGE_2, in adrenocortical tissue is well established. *In vitro* ACTH can induce adrenocortical PG synthesis (Laychock and Rubin, 1975) and indomethacin, which inhibits adrenal PG synthesis (Thompson and Hedge, 1981), can inhibit ACTH-stimulated glucocorticoid secretion (Honn and Chavin, 1977; Spät *et al.*, 1977). *In vitro* reduced PG synthesis inhibits corticosteroid secretion in response to ACTH but not dibutyryl cAMP (Gallant and Brownie, 1973) and indomethacin exerts a direct effect on the adrenal, decreasing responsiveness to acute elevations in ACTH (Thompson and Hedge, 1981). In the latter study, despite decreased responsiveness to ACTH, basal concentrations of corticosterone were increased by the indomethacin treatment, probably secondary to elevated plasma ACTH concentrations. Thompson and Hedge (1981) suggested that the site of action of PGs was at, or before, the activation of adenylate cyclase by ACTH. PGE_2 may therefore act to increase ACTH receptors or to improve the coupling of the receptor to the adenylate cyclase, possibly at the level of the G_s protein, or via the IP_3/Ca^{2+} receptor pathway.

By infusing PGE_2 and $PGF_{2\alpha}$ into fetal lambs on days 125–130 of pregnancy, Louis *et al.* (1976) examined the possibility that PGs might influence fetal adrenal production of corticosteroids. At this stage of gestation, the fetal adrenal responds poorly to short-term infusions of $ACTH_{1-24}$; PGE_2 provoked a

threefold increase in fetal plasma cortisol, whereas a similar amount of $PGF_{2\alpha}$ had no effect. The capacity of the placenta to synthesize PGs may therefore be associated with maturation of the fetal adrenal gland. There is a progressive increase in PGE_2 concentration in the fetal circulation (Fowden *et al.*, 1987), suggesting that the stimulus to fetal adrenal function may come from the placenta (Thorburn *et al.*, 1989). Liggins *et al.* (1982) confirmed the results of Louis *et al.* (1976) and showed that the effect of PGE_2 was not significantly different in intact and hypophysectomized fetuses, eliminating the possibility that pituitary peptides were involved. However, PGE_2 may release ACTH from the ovine placenta as it does from the human placenta (Petraglia *et al.*, 1987).

Specific PGE_2 receptors have been identified in the adrenal membranes of adult humans and sheep, and they stimulate adenylate cyclase activity (Dazord *et al.*, 1974). It is not known whether PGE_2 binding sites are present in ovine fetal membranes, but PGE_2 (10^{-5} M) did not stimulate adenylate cyclase in adrenal membranes of the fetal or newborn (3 days) lamb (Durand *et al.*, 1981b). PGE_2 presumably exerts its action on the fetal adrenal via membrane receptors linked to the adenylate cyclase system (Rice and Thorburn, 1988; Thorburn *et al.*, 1988; Thorburn and Rice, 1990; Thorburn, 1991). Coleman *et al.* (1985) have postulated that PGE_2 can act through three types of receptors, which they have designated, EP_1, acting via the IP^3/Ca^{2+} pathway (present in contractile tissues like smooth muscle); EP_2, via the adenylate cyclase pathway; and EP_3, also acting via the adenylate cyclase pathway but in an inhibitory mode. EP_1 receptors may therefore be present on the fetal adrenal between days 100 and 130 of gestation, and the infusion of PGE_2 may induce a steroidogenic response by stimulating the EP_1 receptors and activating the IP_3/Ca^{2+} pathway. These putative EP_1 receptors and the type I ACTH receptor may be linked to the same pathway. PGE_2, but not cAMP or LH, can stimulate P_4 synthesis in isolated ovine binucleate cells (Wango *et al.*, 1988), suggesting that PGE_2, may act in an autocrine/paracrine fashion to stimulate placental P_4 synthesis, probably by an IP_3/Ca^{2+} pathway. Further studies will be needed to determine whether the IP_3/Ca^{2+} pathway develops earlier than the adenylate cyclase pathway in the fetal adrenal.

10.11 THE DEVELOPMENT OF GLUCOCORTICOID FEEDBACK ON THE FETAL HPA AXIS

Perhaps the key element in the initiation of parturition in sheep is that cortisol must reach sufficiently high concentrations in fetal plasma and be maintained for long enough (36–48 h) to induce high 17α-hydroxylase activity in the placenta (Anderson *et al.*, 1975; France *et al.*, 1988; Mason *et al.*, 1989; Thorburn, 1991; Thorburn *et al.*, 1991). The increased activity of this enzyme causes an increase in the metabolism of P_5 away from P_4 to oestrogens, a profound increase in the oestrogen–progesterone ratio, a steep increase in PGF release, and thereby triggers parturition (Thorburn *et al.*, 1991). Analysis of hormone profiles (Thorburn *et al.*, 1989) of ewes entering term labour (Magyar *et al.*, 1980a; Elsner *et al.*, 1980; Nathanielsz *et al.*, 1982) showed that P_4 concentrations began to decrease rapidly 3.5 days (mean figure) before delivery and this coincided with fetal cortisol concentrations of about 30–40 ng/ml (Magyar *et al.*, 1980a). These levels are achieved only during the preparturient surge of cortisol (Thorburn *et al.*, 1989).

To achieve such high cortisol concentrations it is essential that high plasma $ACTH_{1-39}$ values are maintained during the preparturient cortisol surge. This raises the question of why, during the days preceding parturition, cortisol does not feedback on the hypothalamus and pituitary to inhibit the release of $ACTH_{1-39}$. Earlier in gestation (117–131 days' gestation), the fetal

hypothalamic–pituitary unit is very sensitive to cortisol (Wood, 1986) but, in the last few days of fetal life, the negative feedback sensitivity is apparently absent, allowing the preparturient rise in cortisol to be maintained (Wood, 1987, 1988). It has been suggested that the maintenance of ACTH involves some neural pathways which are not subject to feedback inhibition by cortisol (Wood, 1988) or that some other powerful stimulus to CRF release may override the negative-feedback effects of cortisol. Placental PGE_2, which is present in high concentration in the fetal circulation in the days preceding parturition, may provide such a stimulus (Thorburn, 1991).

The studies of Wintour *et al.* (1980) in adrenalectomized fetuses suggested that the hypothalamic drive to the pituitary increases from 120–125 days of gestation, leading to a progressive increase in ACTH and β-endorphin concentrations. This finding clearly demonstrated the presence of an hypothalamic drive to the pituitary causing the release of increasing amounts of ACTH into the fetal circulation. The full extent of the stimulus was only revealed when the inhibitory effect of cortisol was removed by adrenalectomy. In attempting to unravel the mechanisms involved in the initiation of parturition in sheep, it would seem essential to determine the nature of the stimulatory input into the hypothalamus.

After fetal adrenalectomy at 115 days' gestation, and hence removal of the inhibitory action of cortisol on the hypothalamus and pituitary, POMC mRNA levels in the fetal pituitary are significantly higher than in intact fetuses of the same gestational age (135 days) (McMillen *et al.*, 1990).

The high levels of pituitary POMC mRNA found in adrenalectomized fetuses suggested that, in addition to the stimulus to the hypothalamus from the central 'trigger', the hypothalamus and pituitary are released from the negative feedback effect of cortisol making them more responsive to any stimulatory inputs. The high plasma concentrations of PGE_2 found in adrenalectomized fetuses (Hollingworth *et al.*, 1990a) may provide a further stimulus for the release of CRH and AVP thus helping to maintain the high levels of ACTH found in these fetuses. To what extent the increase in plasma ACTH levels in adrenalectomized fetuses (Wintour *et al.*, 1980) is due to the progressive increase in fetal plasma concentrations of PGE_2, rather than stimulation from some central 'trigger' or from the removal of cortisol inhibition, is unknown. The cause of the increase in placental PGE_2 production is also unknown, although it has been suggested (Thorburn *et al.*, 1988; Thorburn, 1991) that it is related in some way to the rapid increase in fetal growth at this time.

Chromatographic analyses of plasma samples from adrenalectomized fetuses showed that $ACTH_{1-39}$ was present in substantial amounts much earlier in gestation (119 days was the earliest sample examined) than in plasma from control fetuses (Hollingworth *et al.*, 1990a; Thorburn *et al.*, 1991). The administration of cortisol to these adrenalectomized fetuses selectively reduced values of $ACTH_{1-39}$ (Hollingworth *et al.*, 1990b) but not those of high or low molecular weight precursors. The proposed model suggests that the $ACTH_{1-39}$ released from the CRF target cells resides in vesicles within those cells (Schwartz, 1990). Furthermore, the CRF responsive cells are the likely site for the release of pulses of $ACTH_{1-39}$ and a paracrine factor that acts to inhibit the secretion of ACTH (HMW forms) from other cells (Schwartz, 1990).

Parkes *et al.* (1988), using an IRMA to measure $ACTH_{1-39}$ specifically reported a parturient increase (fivefold) in $ACTH_{1-39}$ but found no evidence of a rise in $ACTH_{1-39}$ when the increase in fetal cortisol concentrations is expected. The increase in $ACTH_{1-39}$ concentrations may start about 126–130 days of gestation (Hollingworth *et al.*, 1990a) or far earlier (Norman *et al.*, 1985)

but adenylate cyclase activity in the fetal membrane and the major increase in fetal cortisol concentrations develop after 135–137 days of gestation.

A trophic drive to the HPA axis therefore appears to occur relatively early in gestation but is revealed only when the negative-feedback inhibition by cortisol is removed by fetal adrenalectomy. CRF is present in the hypothalamus quite early (115–120 days) but is not released until much later in response to cortisol.

If $ACTH_{1-39}$ is not released in significant amounts until late in gestation (e.g. $\geqslant$135 days' gestation), it is reasonable to ask what causes the initial increase in fetal cortisol levels. The present evidence suggests that HMW POMC species are present in the fetal circulation earlier than is $ACTH_{1-39}$ (Jones, 1983; Hollingworth *et al.*, 1990a; Thorburn *et al.*, 1991). Although they are considered to be only weakly steroidogenic, these peptides may be present in sufficient quantities to initiate a small increase in cortisol secretion from the fetal adrenal. PGE_2 secreted by the placenta may also play a key role (in concert with the HMW species) in stimulating cortisol secretion (Louis *et al.*, 1976; Liggins *et al.*, 1982). When placental PGE_2 release is stimulated by hypoxaemia secondary to restricting placental blood flow, cortisol concentrations are increased and maintained at high levels (Hooper *et al.*, 1990). Thus, together or individually, PGE_2 and the HMW species of POMC may initiate the increase in fetal cortisol levels, acting through a cAMP-independent pathway (section 10.7). This may be enhanced by a decrease in the plasma concentrations of the putative inhibitor proposed by Durand *et al.* (1982a). The gradual increase in fetal cortisol concentrations therefore plays an important part in initiating the maturation of key organ systems of the body (Liggins *et al.*, 1979; Thorburn, 1987) while delaying parturition, by inhibiting the release of $ACTH_{1-39}$, until the maturation process is complete.

Our recent results (Carr *et al.*, 1992), using an IRMA, show a progressive increase in $ACTH_{1-39}$ concentrations from early in the last third of gestation whereas the concentration of the HMW ACTH species remained unchanged, suggesting that the efficacy of the cortisol feedback decreases with time or the stimulus for the release of ACTH progressively increases or both.

Recent studies have clearly demonstrated the ability of cortisol to inhibit ACTH release in adrenalectomized fetuses (Hollingworth *et al.*, 1990b). The administration of cortisol to adrenalectomized fetuses, in amounts sufficient to achieve parturient levels of cortisol in fetal plasma, caused a dramatic decrease in $ACTH_{1-39}$ concentrations to very low levels. In normal fetuses at term, with the same cortisol concentrations, plasma $ACTH_{1-39}$ concentrations were found to be only modestly reduced (S.A. Hollingworth, personal communication, 1993). These results suggest that, in intact fetuses, some positive stimulus opposes the inhibitory action of cortisol on the hypothalamus. This positive stimulus must be independent of the 'trigger' which is present and active in both adrenalectomized and intact fetuses. Since fetal plasma PGE_2 concentrations are high at this time (Fowden *et al.*, 1987; Hollingworth, 1993) and PGE_2 is known to be a potent stimulator of ACTH release (Hollingworth, 1990a), it is possible that PGE_2, derived mainly from the placenta (Fowden, 1987), may be the stimulus that maintains the $ACTH_{1-39}$ concentrations high in the face of high circulating levels of cortisol in normal fetuses at term.

To summarize the above discussion, there appears to be a stimulatory drive to the fetal hypothalamus from a putative 'trigger' mechanism, the site and nature of which is unknown, although it is thought to reside in the central nervous system. This drive initially succeeds in producing only a small increase in fetal plasma $ACTH_{1-39}$ levels (Norman and Challis, 1987a; Carr *et al.*, 1992; Deayton *et al.*, 1993) and in initiating a small increase in fetal

plasma concentrations of cortisol starting about 120–125 days' gestation. This early activation of the HPA axis may be enhanced by the action of PGE_2 acting at the hypothalamus to stimulate the release of CRF and AVP, and thus ACTH and at the adrenal cortex to increase steroidogenesis. It is also possible that the 'trigger' may reside in the placenta and its action on the HPA axis is mediated by PGE_2. The activation of a placental trigger may be related to the growth of the fetus and the metabolic demands placed on the placenta (Thorburn, 1991; Thorburn *et al.*, 1988).

In the absence of adenylate cyclase (type 2) receptors in the fetal adrenal membranes before 137–140 days' gestation, $ACTH_{1-39}$ and PGE_2 may act via type 1 IP_3 receptors to initiate a small, but progressive increase in fetal cortisol concentrations. The increasing levels of cortisol in the fetal circulation exert a positive feedback action on the fetal adrenal cortex by increasing 17α-hydroxylase activity and enhancing the synthesis of further cortisol (see Challis and Brooks, 1989). The increasing cortisol levels may also influence the processing of POMC favouring the secretion of $ACTH_{1-39}$. In the light of recent data (S.R. Crosby, personal communication, 1993), the presence of high concentrations of HMW ACTH species before 140 days' gestation would reduce the responsiveness of the adrenal cortex to $ACTH_{1-39}$.

CRF is present in the fetal hypothalamus before 115–120 days' gestation, and can be released by the fetal infusion of PGE_2, but in the normal fetal sheep, it is not released in significant amounts until much later because of the inhibitory action of cortisol. The low concentrations of cortisol in fetal plasma between 120–140 days play an important role in preparing the fetus for postnatal life by initiating the maturation of key organ systems (Liggins *et al.*, 1979; Thorburn, 1987).

10.12 POSSIBLE HETEROGENEITY OF FETAL PITUITARY CORTICOTROPHS

The above proposal requires the differential regulation of the secretion of the various POMC species, favouring the secretion of the weakly steroidogenic POMC species initially and the more active $ACTH_{1-39}$ later. It was thought that the pituitary corticotrophs were a homogeneous population of cells that responded similarly to hypothalamic factors such as CRF and AVP. However, different cell populations appear to exhibit different secretory patterns in response to CRF, AVP or in the absence of a secretogogue (Schwartz and Vale, 1988; Schwartz, 1990; Schwartz *et al.*, 1991). Pradier *et al.* (1988) noted that in the adult sheep intermediate molecular weight forms of immunoreactive ACTH are secreted by the pituitary in response to AVP, whereas $ACTH_{1-39}$ is preferentially secreted in response to CRF. Thorburn *et al.* (1991) have suggested that the HMW species of POMC may be preferentially secreted in response to AVP by AVP target cells which could also secrete ACTH via a constitutive pathway (Schwartz *et al.*, 1991) and secrete a stimulatory paracrine factor which acts on adjacent corticotrophs (Schwartz, 1990). The putative stimulatory factor may be the lipoxygenase derivative (possibly 12-HPETE) mentioned above (section 10.10.1). This model could be used to explain changes in the fetal pituitary with increasing gestational age and in POMC peptide concentrations in the fetal circulation. The predominance of the HMW species of POMC in the fetal circulation before 135–139 days of gestation may be due to the earlier appearance of the AVP target cells in the fetal pituitary and, possibly, the secretion of AVP by the fetal hypothalamus during this time. This suggestion would be consistent with the findings of Norman and Challis (1987a,b) that the fetal pituitary is sensitive to AVP stimulation (as judged by an increase in plasma ACTH levels) between

days 100 and 130 of gestation but not at days 135–140.

Thorburn *et al.* (1991) have suggested that the late appearance of $ACTH_{1-39}$ in the fetal circulation may be due to the late appearance of CRF target cells or the suppression of CRF release by the low levels of cortisol in the fetal circulation. Fetal adrenalectomy experiments (Thorburn *et al.*, 1991) would favour the latter suggestion. The initial stimulatory drive from the hypothalamus to the fetal pituitary may therefore be AVP before subsequent release of CRF.

10.13 WHAT CONTROLS THE RELEASE OF $ACTH_{1-39}$?

The next critical step in the sequence of events leading to parturition in sheep is an increase in the concentrations of $ACTH_{1-39}$ in fetal plasma. There is some dispute about the timing of this step, but recent data indicate that a **significant** increase in $ACTH_{1-39}$ concentration does not occur before day 135 of gestation (Parkes *et al.*, 1988; Hollingworth *et al.*, 1990a; Deayton *et al.*, 1993) consistent with the timing of the rapid increase in ACTH receptors and adenylate cyclase activity in the fetal adrenal membranes, both of which might be expected since ACTH is known to increase its own adenylate cyclase receptors. These changes would explain the dramatic increase in fetal cortisol concentrations in the 4–5 days before delivery. The most obvious cause of this increase in $ACTH_{1-39}$ concentration is fetal plasma is an increase in the secretion of CRF by the hypothalamus. When feedback inhibition of the hypothalamus by cortisol is removed by fetal adrenalectomy, pituitary POMC mRNA levels increase markedly (McMillen *et al.*, 1990) and there is a marked increase in POMC peptides in the fetal circulation and the premature release of $ACTH_{1-39}$. If the stimulus for $ACTH_{1-39}$ secretion was coming simply from increased secretion of CRF, it might be expected that pituitary POMC mRNA levels would increase in the days before delivery, but McMillen *et al.* (1988) found reduced POMC mRNA levels after 140 days of gestation. However, when fetal pituitaries were removed from ewes in labour, Yang *et al.* (1991) found an increase in some ewes although there was considerable variability between pituitaries.

A potential difficulty in the interpretation of these results is that total anterior pituitary mRNA was measured; if only a subpopulation of the corticotrophs is responsible for secreting most of the circulating $ACTH_{1-39}$, these measurements may not reflect the change in POMC mRNA levels in these different cell populations. However, the decrease in the POMC mRNA levels observed by McMillen *et al.* (1988) is consistent with cortisol exerting a negative feedback (in the slow domain, Dallman *et al.*, 1987) at the pituitary level. Ozolins *et al.* (1990) have also demonstrated with HPD fetuses that cortisol can exert a negative feedback at the pituitary level. There are several possible reasons for the decrease in POMC mRNA levels in the pituitary before birth. Firstly, the secretion of CRF from the hypothalamus may actually be decreased rather than increased. Indeed, Brooks and Challis (1988) found that the CRF content of the fetal hypothalamus had decreased by day 140 to the low values observed at day 100, suggesting that high cortisol concentrations were exerting a negative-feedback effect on CRF gene expression, although the measurement of content must be interpreted with caution. The low levels of CRF may indeed be an expression of high turnover and low storage.

It seems likely that the fetal hypothalamus, and particularly the cells which secrete CRF, is subjected to powerful stimulatory influences which counteract the negative-feedback effects of cortisol and permit a continued secretion of CRF. It would seem that PGE_2 can provide a powerful stimulus for CRF and ACTH release in the fetal lamb (Thorburn, 1989; Thorburn *et al.*, 1991) and

high levels of PGE_2 are found in the fetal circulation in the days before parturition (Fowden *et al.*, 1987). PGs are also known to be synthesized locally in the hypothalamus of many species and may regulate the release of pituitary hormones (Hedge, 1977).

The second reason for the low POMC mRNA levels in the anterior pituitary after day 140 is the high fetal cortisol levels. Finally, the relative amount of HMW species of POMC secreted by the fetal pituitary decreases with increasing gestation (S.A. Hollingworth, personal communication) perhaps due to low levels of POMC mRNA in the putative AVP target cells rather than the CRF target cells. This suggestion would be consistent with the observation by Norman and Challis (1987a) that the fetal pituitary becomes less responsive to AVP by 135–140 days of gestation. Low levels of POMC mRNA in AVP target cells may be secondary to a decrease in the secretion of AVP by the fetal hypothalamus late in gestation or to an increased release of the inhibitor from CRF target cells.

Keeping all these reservations in mind, it is important to consider how the pituitary secretes increasing amounts of $ACTH_{1-39}$ despite decreasing amounts of POMC mRNA. There may be more efficient post-translational processing of the POMC mRNA (McMillen *et al.*, 1988) or the CRF target cells may assume a more important role in the days preceding delivery (Thorburn *et al.*, 1991) so that post-translational processing in these cells favours relatively more $ACTH_{1-39}$ and fully processed forms of POMC than HMW species (Schwartz, 1990). Although PGE_2 does not appear to have a direct stimulatory effect on the secretion of pituitary ACTH in the absence of CRF, since it does not increase ACTH secretion in HPD fetuses (S.A. Hollingworth, personal communication), PGE_2 may increase cAMP levels in the pituitary and therefore increase the efficiency of post-transcriptional events. Indeed, both CRF and PGF_2 can act via adenylate cyclase pathways and may act in concert to facilitate this process.

Whether these putative functional cell types correlate with the 'fetal' (AVP target) and 'adult' (CRF target) cell types (Perry *et al.*, 1985; Mulvogue *et al.*, 1986) needs to be determined. Fetal adrenalectomy delays the appearance of 'adult'-type cells, whereas cortisol infusion into the fetus hastens their appearance (Antolovich *et al.*, 1989). The present proposal suggests that various types of pituitary corticotrophs exist in the fetal pituitary during the last third of gestation. It is possible that the CRF target cells are relatively dormant before 135 days, but can be activated by the increased secretion of CRF after fetal adrenalectomy. The fact that the AVP-sensitive cells do not respond to a release of AVP after day 135 of gestation (Norman and Challis, 1987a) does not establish whether AVP target cells have disappeared or have become non-functional because of low mRNA levels resulting from a lack of stimulation by AVP in late gestation or the increased presence of paracrine inhibitors.

With reference to the more efficient post-translational processing of POMC in the near-term fetal pituitary, different processing pathways may occur in the different cell types. However, it is possible that all corticotrophs possess the same pathways and that the redirection of POMC processing occurs in all cells, leading to an increase in the synthesis of $ACTH_{1-39}$ in the face of normal or low levels of POMC mRNA, or that $ACTH_{1-39}$ is preferentially directed to a secretory rather than storage pathway. Several lines of evidence suggest that cortisol itself may influence post-translational processing by favouring the release of $ACTH_{1-39}$ over the HMW species (Thorburn *et al.*, 1991), perhaps by cortisol acting directly on the corticotroph to influence the translational process or by selectively inhibiting the AVP target cells.

In fetal sheep, the rise in plasma cortisol that precedes parturition (Bassett and Thorburn, 1969; Magyar *et al.*, 1980a) is associated

with an increase in the concentration of CBG in fetal plasma (Fairclough and Liggins, 1975; Ballard *et al.*, 1982; Challis *et al.*, 1985), particularly during the second half of gestation, resulting in a threefold increase in CBG capacity by 140 days of gestation (Ballard *et al.*, 1982). The expected rise in fetal CBG capacity did not occur in hydranencephalic, stalk sectioned or hypophysectomized fetuses. However, Fairclough and Liggins (1975) and Challis *et al.* (1985) found that cortisol stimulated the synthesis of its own binding protein. Ballard *et al.* (1982) reported that the concentration of unbound ('free') corticoids was 0.9 ng/ml (12% of total corticoids) at 115 days and did not increase until after 135 days, reaching a maximum level of 18.7 ng/ml (14.5% of total corticoids on the day before spontaneous term delivery), a 16- to 20-fold increase over values at 115–135 days. Fairclough and Liggins (1975) suggested that the early increase in CBG levels protects the fetus from increases in plasma corticoids that could initiate premature labour. Thus, despite apparently high total concentrations of cortisol in fetal plasma, the effective concentration of cortisol exerting a feedback action on the fetal hypothalamus and pituitary is much lower. Since cortisol is thought to induce CBG synthesis in the fetal liver, this mechanism would allow the adrenal to secrete high concentrations of cortisol while the cortisol itself induces its binding protein, thus lowering its 'free' concentration. This system would be particularly effective if the hypothalamus and pituitary were sensitive only to free cortisol whereas the placenta was also sensitive to bound form (i.e. total cortisol). The action of CBG is therefore to lower the efficiency of the negative-feedback system by maintaining lower free cortisol concentrations. The lower fetal cortisol (total) concentrations reported in some species may result from lower CBG levels.

10.14 FETAL HYPOTHALAMUS: ULTRASTRUCTURAL DEVELOPMENT

Specific antibodies to CRF and AVP have been used to locate CRF and AVP neurones in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus of the adult sheep. CRF fibres derived from the neurones projected predominantly to the external lamina of the median eminence (ME), whereas AVP terminals were concentrated in the internal lamina (Levidiotis *et al.*, 1987). CRF neurones were first detected in the fetal PVN at 90 days of gestation, although no connections to the ME were apparent until 105 days. In contrast, AVP neurones were found from 42 days' gestation (earliest age examined); the cell bodies were mainly situated in the SON, but the fibres and terminals were present mainly in the external lamina of the ME and, and the electron microscope level, were closely associated with fenestrated portal vessels (Levidiotis *et al.*, 1987). At later stages there appeared to be redistribution of AVP fibres such that, by 132 days' gestation, AVP projections were more or less equally distributed between the laminae of the ME, whereas in the adult AVP fibres are directed mainly to the internal lamina, in which many are destined for the posterior pituitary. Consistent with the above findings, immunoreactive AVP concentrations in the hypothalamus increase progressively between day 63 and day 138, decrease on day 143 and rise again in post-partum animals (Brieu *et al.*, 1989). The ratio of immunoreactive AVP–CRF was about 5 at days 63 and 123 of gestation, but decreased to 1.2 by term (Brieu *et al.*, 1989). Challis and Brooks (1989) suggested that this ratio reflects the greater efficacy of AVP as a releaser of ACTH in younger fetuses and the decline in potency of AVP relative to CRF with advancing gestation. The early appearance of AVP relative to CRF is consistent with the proposal that AVP may be a significant (and perhaps the major) factor controlling

ACTH release in the ovine fetus before 105 days of gestation (Levidiotis *et al.*, 1987) and probably up to 125–130 days' gestation. It is clear that, despite considerable progress being made in the past few years, more work is needed to explore the many possibilities that have emerged (see review by Challis and Brooks, 1989, for further information on this topic).

10.15 THE 'TRIGGER' FOR PARTURITION

Although the suggestion (Liggins, 1969a) that the fetal hypothalamic–pituitary–adrenal axis played a primary role in the initiation of parturition in sheep has stood the test of time, the 'trigger' for parturition is still uncertain. A single trigger would seem unlikely, because such an important biological system should include a fail-safe mechanism. Some input to the hypothalamus that stimulates the secretion of CRF, leading to activation of the HPA axis, is favoured. The release of AVP appears to precede that of CRF. The placenta could also activate the HPA axis by secretion of PGE_2, although there is no evidence at the present time of placental PGE_2 being the 'trigger' for parturition. The merit of this proposal is that the placenta, being an interface between mother and fetus, responds to insults such as hypoxia, undernutrition and emboli by secreting more PGE_2 (Hooper *et al.*, 1990). The early release of PGE_2 may provide a mechanism for initiating premature labour when the fetus is compromised. Thorburn and Rice (1990) proposed that in normal pregnancies the growth pattern of the fetus may provide the trigger; when the fetus enters its rapid growth phase, PGE_2 secretion by the placenta increases, the HPA axis is activated and cortisol concentrations rise, initiating parturition. One advantage of linking the 'trigger' for parturition with fetal growth (and substrate supply) is that it ensures that the fetus does not outgrow its environment and that it is an appropriate size at term to pass through the birth canal. It provides, therefore, a link between size at birth, fetal maturity and the initiation of parturition.

Cutaneous temperature and pain receptors are active quite early in gestation in the fetal lamb, but the ascending spinothalamic tracts are not functional until around 100 days of gestation (J. Rawson and S.H. Rees, personal communication), and so information will not reach the brain until relatively late in gestation. It can be reasoned that, as the temperature-sensing pathways mature, the fetus becomes aware it is in a hot, humid environment with no means of regulating its temperature and so the 'stress' pathway (the HPA axis) is activated, resulting in its birth. This proposes a physiological mechanism by which the HPA axis might be activated to initiate parturition. This illustrates how the maturation of one physiological pathway may make continued intrauterine life untenable. There are probably many other examples, all of which are likely to activate the HPA axis.

10.16 SUMMARY

Given the evidence of HPA axis activation obtained to date, a possible scheme for the initiation of parturition emerges (Figure 10.3).

Early in the last third of gestation (100–120 days' gestation), the major ACTH species in the fetal circulation are high molecular weight and are probably derived mainly from the AVP target cells in the fetal pituitary. The secretion of HMW species is thought to be stimulated by AVP derived from the fetal hypothalamus. The stimulus for AVP release is either PGE_2, produced locally or in the placenta, or some other stimulatory input into the hypothalamus. The CRF target cells are thought to be relatively quiescent at that time, probably due to a lack of CRF since they can be readily activated to secrete $ACTH_{1-39}$ by adrenalectomizing the fetus and removing the inhibitory feedback effect of cortisol on

the release of CRF from the hypothalamus. The adrenalectomy results suggest that the very low levels of cortisol present before 125 days of gestation must be important for inhibiting CRF release **or** that some other adrenal product suppresses CRF release.

We propose that the major ACTH receptors present in the fetal adrenal before 135–140 days of gestation are type I (IP_3/Ca^{2+}). The HMW species and the $ACTH_{1-39}$ in low concentrations act via the type I receptors to stimulate the fetal adrenal to secrete low levels of cortisol. In some systems activation of IP_3 /Ca^{2+} (type I) receptors can inhibit the activity of the adenylate cyclase (type II) receptors. This may occur in the fetal adrenal and help delay the appearance of the type II receptors until 135 days. PGE_2 may, in concert with the ACTH peptides, stimulate the adrenal type I receptors.

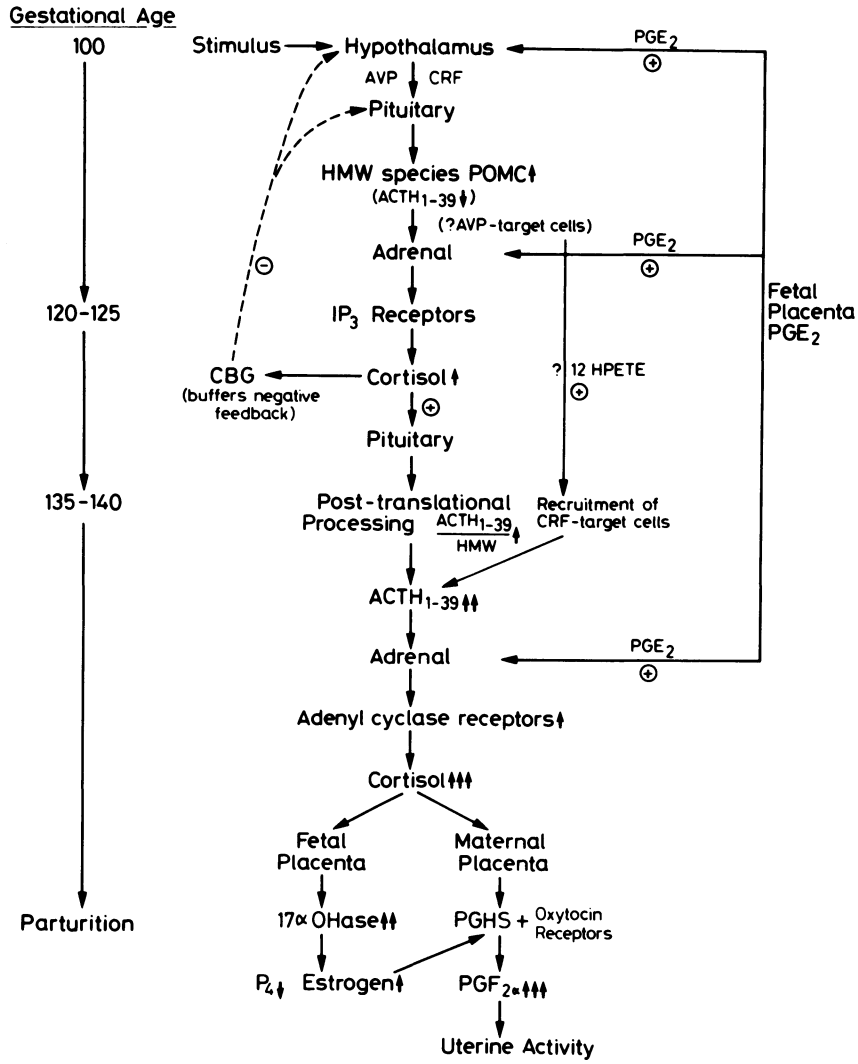

Figure 10.3 Diagrammatic representation of the pathways involved in the initiation of parturition in the sheep.

It is proposed that, with continued stimulation of the AVP target cells, a potent intercellular stimulator is produced which acts on the CRF target cells, rendering them more sensitive to CRF stimulation. Thus, about day 125, perhaps without any increased secretion of CRF, the CRF-responsive cells secrete increasing amounts of $ACTH_{1-39}$ and a substance which is thought to inhibit the activity of the AVP target cells. In this way, the major functional cell type in the fetal pituitary switches gradually from the AVP target cells to the CRF target cells. With the increasing secretion of $ACTH_{1-39}$ by the CRF target cells, increasing numbers of type II (adenylate cyclase) receptors appear in the fetal adrenal from day 135 onwards. The increase in type II receptors leads to a major increase in cortisol production by the fetal adrenal during the last 4–6 days before delivery.

The increasing levels of cortisol exert a negative feedback on the hypothalamus and pituitary. The cortisol inhibition of CRF is countered to a large extent by the stimulus of PGE_2. While the effect of cortisol on the CRF target cells is likely to inhibit transcription of the POMC gene, it may enhance post-transcriptional events leading to secretion of greater amounts of $ACTH_{1-39}$. The increasing levels of cortisol also augment 17α- hydroxylase activity in the fetal adrenal, enhancing the synthesis of further cortisol. Cortisol also stimulates the secretion of CBG from the fetal liver, lowering the concentration of free cortisol and reducing the negative-feedback effects of cortisol.

The progressive increase in the plasma concentrations of $ACTH_{1-39}$ results in a major increase in fetal plasma cortisol values over the last 4–6 days before delivery. The high levels of cortisol induce high levels of 17α-hydroxylase activity in the placenta and lead to a dramatic decrease in maternal P_4 concentrations and an increase in oestrogen concentrations. Finally, the change in the E–P_4 ratio stimulates the release of $PGF_{2\alpha}$ and the initiation of labour.

The foregoing is but one possible model for the initiation of parturition in the sheep. The fetal HPA axis is only the final common pathway leading to parturition, and the maturation of this pathway takes place in an orderly fashion over a relatively long time, at least the last third of gestation. In the fetus, as in the adult, the afferent information influencing the HPA axis can be derived from many sources and can act at several levels. It seems unlikely that a single 'trigger' for the initiation of parturition will be found.

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REFERENCES

Al Dujaili, E.A., Hope, J., Eshivarez, F.E., Lowry, P.J. and Edwards, C.R. (1981) Circulating human pituitary pro-gamma-melanotropin enhances the adrenal response to ACTH. *Nature*, **291**, 156–159.

Alexander, D.P., Britton, H.G., James, V.T.H., Nixon, D.A., Parker, R.A., Wintour, E.M. and Wright, R.D. (1968) Steroid secretion by the adrenal gland of foetal and neonatal sheep. *Journal of Endocrinology*, **40**, 1–13.

Alexander, D.P., Britton, H.G., Nixon, D.A., Ratcliffe, J.G. and Redstone, D. (1973) Corticotrophin and cortisol concentrations in the plasma of the chronically catheterised sheep fetus. *Biology of the Neonate*, **23**, 184–192.

Anderson, A.B.M, Pierrepoint, C.G., Jones, C.T., Griffiths, K. and Turnbull, A.C. (1970) Steroid biosynthesis *in vitro* by foetal and adult sheep adrenal tissue. *Journal of Reproduction and Fertility*, **22**, 99–107.

Anderson, A.B.M., Pierrepoint, C.G., Griffiths, K. and Turnbull, A.C. (1972) Steroid metabolism in the adrenals of fetal sheep in relation to natural and corticotrophin-induced parturition. *Journal of Reproduction and Fertility*, Suppl. 16, 25–37.

Anderson, A.B.M., Flint, A.P.F. and Turnbull, A.C. (1975) Mechanism of action of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. *Journal of Endocrinology*, **66**, 61–70.

Andersson, B. and Leksell, L.G. (1975) Effects on fluid balance of intraventricular infusions of prostaglandins E_1. *Acta Physiologica Scandinavica*, **93**, 286–288.

Antolovich, G.C., Perry, R.A., Trahair, J.F., Silver, M. and Robinson, P.M. (1989) The development of corticotrophs in the fetal sheep pars distalis: the effect of adrenalectomy or cortisol infusion. *Endocrinology*, **124**, 1333–1339.

Ballard, P.L., Kitterman, J.A., Bland, R.D. Clyman, R.I., Gluckman, P.D., Platzer, A.C.G., Kaplan, S.L. and Grumbach, M.M. (1982) Ontogeny and regulation of corticosteroid binding globulin capacity in plasma of fetal and newborn lambs. *Endocrinology*, **110**, 359–366.

Bassett, J.M. and Thorburn, G.D. (1969) Foetal plasma corticosteroids and the initiation of parturition in the sheep. *Journal of Endocrinology*, **44**, 285–286.

Bassett, J.M. and Thorburn, G.D. (1973) Circulating levels of progesterone and corticosteroids in the pregnant ewe and its foetus. In *Endocrinology of Pregnancy and Parturition – Experimental Studies in the Sheep*. (ed. C.G. Pierrepoint) Alpha Omega Alpha Publications, Cardiff, UK, pp. 126–140.

Basson, P.A., Morgenthal, J.C., Bilbrough, R.B., Marais, J.L., Kruger, S.P. and Van Der Merwe, J.L. (1969) 'Grootlamsiekte', a specific syndrome of prolonged gestation in sheep caused by a shrub *Salsola tuberculata*. *Onderstepoort Journal of Veterinary Research* **36**, 59.

Berl, T. and Schrier, R.W. (1973) Mechanism of effect of prostaglandin E_1 on renal water excretion. *Journal of Clinical Investigation*, **52**, 463–471.

Binns, W., Lynn, J.F., Shupe, J.L. and Everett, M.S. (1963) A congenital cyclopian-type malformation in lambs induced by maternal ingestion of a range plant, Veratrum californicum. *American Journal of Veterinary Research*, **4**, 1164–1175.

Binns, W., James, L.F. and Shupe, J.L. (1964) Toxicosis of Veratrum californicum in ewes and its relationship to a congenital deformity in lambs. *Annals of the New York Academy of Science*, **111**, 571–576.

Brieu, V. and Durand, P. (1987) Changes in the ratio of bioactive to immunoreactive adrenocorticotropin-like activity released by pituitary cells from ovine fetuses and lambs. *Endocrinology*, **120**, 936–940.

Brieu, V., Tonon, M.C., Lutz-Bucher, B. and Durand, P. (1989) Corticotropin-releasing factor-like immunoreactivity, arginine vasopressin-like immunoreactivity and ACTH-releasing bioactivity in hypothalamic tissue from fetal and neonatal sheep. *Neuroendocrinology*, **49**, 164–168.

Brooks, A.N. and Challis, J.R.G (1988) Regulation of the hypothalamic–pituitary–adrenal axis in birth. *Canadian Journal of Physiology and Pharmacology*, **66**, 1106–1112.

Brown, E.H., Coghlan, J.P., Hardy, K.J. and Wintour, E.M. (1978) Aldosterone, corticosterone, cortisol, 11-deoxycortisol and 11-deoxycorticosterone concentrations in the blood of chronically cannulated ovine foetuses: effect of ACTH. *Acta Endocrinologica (Copenhagen)*, **88**, 364–374.

Cabalum, T.C., Oakes, G.K., Jansen, C.A.M., Yu, H.K., Hammer, T., Buster, J.E. and Nathanielsz, P.W. (1982) Induction of parturition in sheep by low dose intrafetal infusion of synthetic adrenocorticotropin-(1–24) at 120–130 days gestation. *Endocrinology* **110**, 1408–1415.

Carr, G., Jacobs, R., Young, R., Schwartz, J. and Thorburn, G.D. (1992) Hypothalamic-pituitary–adrenal axis in fetal sheep: axis development and synergism between AVP and CRF (abstract). *Proceedings of the Australian Physiological and Pharmacological Society*, p. 68.

Cathiard, A.M., Crozat, A., Durand, P. and Saez, J.M. (1985) *In vitro* spontaneous and adrenocorticotropin-dependent maturation of the steroidogenic pathway of ovine fetal adrenal cells. *Endocrinology*, **116**, 585–590.

Challis, J.R.G. and Brooks, A.N. (1989) Maturation and activation of hypothalamic-pituitary–adrenal function in fetal sheep. *Endocrine Reviews*, **10**, 182–204.

Challis, J.R.G., Harrison, F.A., Heap, R.B., Horton, E.W. and Poyser, N.L. (1982a) Possible role of oestrogens in the stimulation of $PGF_{2\alpha}$ output at the time of parturition in sheep. *Journal of Reproduction and Fertility*, **30**, 485–488.

Challis, J.R.G., Manchester, E.L., Mitchell, B.F. and Patrick, J.E. (1982b) Activation of adrenal function in fetal sheep by the infusion of adrenocorticotropin (ACTH) to the fetus *in utero*. *Biology of Reproduction*, **27**, 1026–1032.

Challis, J.R.G, Mitchell, B.F. and Lye, S.J. (1984)

Activation of fetal adrenal function. *Journal of Developmental Physiology*, **6**, 93–105.

Challis, J.R.G, Nancekievill, E.A. and Lye, S.J. (1985) Possible role of cortisol in the stimulation of cortisol binding capacity in the plasma of fetal sheep. *Endocrinology* **116**, 1139–1144.

Challis, J.R., Fraher, L., Oosterhuis, J., White, S.E. and Bocking, A.D. (1989) Fetal and maternal endocrine responses to prolonged reductions in uterine blood flow in sheep. *American Journal of Obstetrics and Gynecology*, **160**, 926–932.

Chatelain, A. and Dupouy, J.P. (1980) Activité biologique et immunoreactivité des différentes formes moleculaires de l'ACTH hypophysaire chez le foetus de rat. *Journal of Physiology (Paris)*, **76**, 257–263.

Coleman, R.A., Humphrey, P.P.A and Kennedy, I. (1985) Prostanoid receptors in smooth muscle: further evidence for a proposed classification in, *Trends in Autonomic Pharmacology*, Vol. 3, (eds S. Kalsner and F. Taylor), Taylor & Francis, London, pp. 35–49.

Comline, R.S. and Silver, M. (1961) The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep. *Journal of Physiology*, **156**, 424–444.

Comline, R.S., Nathanielsz, P.W., Paisey, R.B. and Silver, M. (1970) Cortisol turnover in the sheep foetus immediately prior to parturition. *Journal of Physiology*, **210**, 141P.

Connors, M.H. and Liggins, G.C. (1980) Adrenocortical responses to adrenocorticotrophin in the hypophysectomized ovine fetus. *Journal of Developmental Physiology*, **2**, 183–190.

Crosby, S.R., Stewart, M.F., Ratcliffe, J.G. and White, A. (1988) Direct measurement of the precursors of adrenocorticotropin in human plasma by two-site immunoradiometric assay. *Journal of Clinical Endocrinology and Metabolism*, **67**, 1272–1277.

Crosby, S.R., Stewart, M.F., Farrell, W.E., Gibson, S. and White, A. (1989) Comparison of ACTH and ACTH precursor peptides secreted by human pituitary and lung tumour cells *in vitro*. *Journal of Endocrinology*, **125**, 147–152.

Dallman, M.F., Akana, S.F., Cascio, C.S., Darlington, D.N., Jacobson, L. and Levin, N. (1987) Regulation of ACTH secretion: variations on a theme of B. *Recent Progress in Hormone Research*, **43**, 113–167.

Dazord, A., Morera, A.M., Berbrand, J. and Saez, J.M. (1974) Prostaglandin receptors in human and ovine adrenal glands: binding and stimulation of adenylcyclase in subcellular preparations. *Endocrinology*, **95**, 352–359.

Deayton, J.M., Young, I.R., Hollingworth, S.A., White, A., Crosby, S.R. and Thorburn, G.D. (1993) Effect of late hypothalamo-pituitary disconnection on the development of the HPA axis in the ovine fetus and the initiation of parturition. *Journal of Neuroendocrinology*, (in press).

Drost, M. and Holm, L.W. (1968) Prolonged pregnancy in ewes after foetal adrenalectomy. *Journal of Endocrinology*, **40**, 293–296.

Drost, M., Kumagai, L.F. and Guzman, M. (1973) Sequential foetal–maternal plasma cortisol levels in sheep. *Journal of Endocrinology*, **56**, 483–492.

Durand, P. (1979) ACTH receptor levels in lamb adrenals at late gestation and early neonatal stages. *Biology of Reproduction*, **20**, 837–845.

Durand, P., Cathiard, A.M., Locatelli, A., Dazord, A. and Saez, J.M. (1981a) Spontaneous and ACTH-induced maturation of the responsiveness of ovine fetal adrenal cells to *in vitro* stimulation by $ACTH_{1-24}$ and cholera toxin. *Endocrinology*, **109**, 2117–2123.

Durand, P., Cathiard, A.M., Morera, A.M., Dazord, A. and Saez, J.M. (1981b) Maturation of adrenocorticotropin-sensitive adenylate cyclase of ovine fetal adrenal during late pregnancy. *Endocrinology*, **108**, 2114–2119.

Durand. P., Locatelli, A., Cathiard, A.M., Dazord, A. and Saez, J.M. (1981c) ACTH induction of the maturation of the ACTH-sensitive adenylate cyclase system in the ovine fetal adrenal. *Journal of Steroid Biochemistry*, **15**, 445–448.

Durand, P., Cathiard, A.M. and Saez, J.M. (1982a) *in vitro* maturation of ovine fetal adrenal cells adenylate cyclase: corticotropin-dependent and independent development of the response to corticotropin. *Biochemical and Biophysical Research Communications*, **106**, 8–15.

Durand, P., Cathiard, A.M., Locatelli, A. and Saez, J.M. (1982b) Modifications of the steroidogenic pathway during spontaneous and adrenocorticotropin-induced maturation of ovine fetal adrenal. *Endocrinology*, **110**, 500–505.

Durand, P., Cathiard, A.M., Seidah, N.G., Chretien, M. and Saez, J.M. (1984a) Effects of proopiomelanocortin-derived peptides, methionine-enkephalin and forskolin on the maturation of ovine fetal adrenal cells in culture. *Biology of Reproduction*, **31**, 694–704.

Durand, P., Cathiard, A.M. and Saez, J.M. (1984b) *in vitro* maturation of steroidogenic capacity of

ovine fetal and neonatal adrenal cells. *Endocrinology*, **114**, 1128–1134.

Durand, P., Cathiard, A.M., Locatelli, A. and Saez, J.M. (1985a) Effects of fetal hypophysectomy and the *in vitro* treatment by pituitary extracts on the maturation of cultured ovine fetal adrenal cells. *Endocrinology*, **116**, 578–584.

Durand, P., Cathiard, A.M. and Saez, J.M. (1985b) Maturation of the ovine fetal adrenal gland: *in vivo* and *in vitro* studies. in, *The Endocrine Physiology of Pregnancy and the Peripartal Period*. (eds R.B. Jaffe and S. Dell'Acqua) Serono Symposia, Siena, Italy, Raven Press, New York, pp. 31–52.

Durand, P., Cathiard, A.M., Naaman, E., Brieu, V. and Saez, J.M. (1987) The influence of plasma lipoproteins on steroidogenesis of cultured ovine fetal and neonatal adrenal cells. *Journal of Steroid Biochemistry*, **26**, 425–431.

Ellis, E.F., Shen, J.C., Schrey, M.P., Carchman, R.A. and Rubin, R.P. (1978) Prostacyclin: a potent stimulator of adrenal steroidogenesis. *Prostaglandins*, **16**, 483–490.

Elsner, D.W., Magyar, D.M., Fridshal, D., Eliot, J., Klein, A. Glatz, T., Nathanielsz, P.W. and Buster, J.E. (1980) Time-trend analysis of plasma C_{21} steroids in fetal and maternal sheep during the last 18 days of gestation. *Endocrinology*, **107**, 801–808.

Fairclough, R.J. and Liggins, G.C. (1975) Protein binding of plasma cortisol in the foetal lamb near term. *Journal of Endocrinology*, **67**, 333–341.

Flack, J.D. and Ramwell, P.W. (1972) A comparison of the effects of ACTH, cyclic AMP, dibutyryl cyclic AMP and PGE_2 on corticosteroidogenesis *in vitro*. *Endocrinology*, **90**, 371–377.

Fowden, A.L., Harding, R., Ralph, M.M. and Thorburn, G.D. (1987) The nutritional regulation of plasma prostaglandin E concentrations in the fetus and pregnant ewe during late gestation. *Journal of Physiology (London)*, **394**, 1–12.

France, J.T., Magness, R.R., Murry, B.A., Rosenfeld, C.R. and Mason, J.I. (1988) The regulation of ovine placental steroid 17 alpha-hydroxylase and aromatase by glucocorticoid. *Molecular Endocrinology*, **2**, 193–199.

Gallant, S. and Brownie, A.C. (1973) The *in vivo* effect of indomethacin and prostaglandin E_2 on ACTH and DBCAMP-induced steroidogenesis in hypophysectomized rats. *Biochemical and Biophysical Research Communications*, **55**, 831–836.

Gallo-Payet, N. and Payet, M.D. (1989) Excitation–secretion coupling: involvement of potassium channels in ACTH-stimulated rat adrenocortical cells. *Journal of Endocrinology*, **120**, 409–421.

Gasson, J.C. (1979) Steroidogenic activity of high molecular weight forms of corticotropin. *Biochemistry*, **18**, 4215–4224.

Gasson, J.C. (1980) Forms and activity of high molecular weight adrenocorticotropin in guinea pig. *Peptides (Fayetteville)*, **1**, 223–229.

Glickman, J.A. and Challis, J.R.G. (1980) The changing response pattern of sheep fetal adrenal cells throughout the course of gestation. *Endocrinology*, **106**, 1371–1376.

Hayaishi, O. (1983) Prostaglandin D_2– a neuromodulator, in, *Advances in Prostaglandin Thromboxane and Leukotriene Research*, Vol. 12, (eds B. Samuelsson, R. Paoletti and P. Ramwell) Raven Press, New York, pp. 333–337

Hedge, G.A. (1977) Roles for the prostaglandins in the regulation of anterior pituitary secretion. *Life Sciences*, **20**, 17–34.

Hennessy, D.P., Coghlan, J.P., Hardy, K.J. and Wintour, E.M. (1982) Development of the pituitary–adrenal axis in chronically cannulated ovine fetuses. *Journal of Developmental Physiology*, **4**, 339–352.

Hollingworth, S.A., Ford, V., Young, I.R. and Thorburn, G.D. (1990a) Adrenocorticotrophin$_{(1–39)}$ ($ACTH_{1–39}$) in the ovine fetus: effects of prostaglandin E_2 (PGE_2) infusion and fetal adrenalectomy. *The Placenta and Fetal Membranes* (abstract), Monash Medical Centre, Melbourne p. 11

Hollingworth, S.A., Ford, V., Young, I.R. and Thorburn, G.D. (1990b) The effect of prostaglandin synthesis inhibition and the effect of cortisol in the ovine adrenalectomised fetus. *Australian Physiological and Pharmacological Society*, **21**, 190P.

Hollingworth, S.A., Ford, V., Young, I.R. and Thorburn, G.D. (1990c) The effect of prostaglandin E_2 on the hypothalamic–pituitary–adrenal axis in fetal sheep. *Australian Physiological and Pharmacological Society*, **21**, 120P.

Holm, L.W. (1967) Prolonged pregnancy. *Advances in Veterinary Science*, **11**, 159–205.

Holm, L.W. and Short, R.V. (1962) Progesterone in the peripheral blood of Guernsey and Friesian cows during prolonged gestation. *Journal of Reproduction and Fertility*, **4**, 137–141.

Honn, K.V. and Chavin, W. (1976) Prostaglandin modulation of the mechanism of ACTH action in the human adrenal. *Biochemical and Biophysical Research Communications*, **73**, 164–170.

Honn, K.V. and Chavin, W. (1977) Temporal effects of ACTH, indomethacin and 7-oxa-13-prostynoic acid upon glucocorticoid production by the human adrenal. *Experientia*, **33**, 545–546.

Hooper, S.B., Coulter, C.L., Deayton, J.M., Harding, R. and Thorburn, G.D. (1990) Fetal endocrine responses to prolonged hypoxemia in sheep. *American Journal of Physiology*, **259**, R703–R708.

Ishikawa, S., Saito, T. and Yoshida, S. (1981) The effect of prostaglandins on the release of arginine vasopressin from the guinea pig hypothalamo-neurohypophyseal complex in organ culture. *Endocrinology*, **108**, 193–198.

Jasper, D.E. (1950) Prolonged gestation in the bovine. *Cornell Veterinarian*, **40**, 165–172.

Jones, C.T. (1983) The integration of adrenal and pituitary activity during development, in, *Initiation of Parturition: Prevention of Prematurity*, (4th Ross Conference on Obstetric Research) (eds P.C. MacDonald and J. Porter) Ross Laboratories, Columbus, Ohio, pp. 17–26.

Jones, C.T. and Roebuck, M.M. (1980) ACTH peptides and the development of the fetal adrenal. *Journal of Steroid Biochemistry*, **12**, 77–82.

Jones, C.T., Luther, E., Ritchie, J.W.K and Worthington, D. (1975) The clearance of ACTH from the plasma of adult and fetal sheep. *Endocrinology*, **96**, 231–234.

Jones, C.T., Boddy, K. and Robinson, J.S. (1977a) Changes in the concentration of adrenocorticotropin and corticosteroid in the plasma of fetal sheep in the latter half of pregnancy and during labour. *Journal of Endocrinology*, **72**, 293–300.

Jones, C.T., Boddy, K., Robinson, J.S. and Ratcliffe, J.G. (1977b) Developmental changes in the responses of the adrenal glands of the fetal sheep to endogenous adrenocorticotrophin as indicated by the hormone responses to hypoxaemia. *Journal of Endocrinology*, **72**, 279–292.

Jones, C.T., Johnson, P., Kendall. J.Z., Ritchie, J.W.K. and Thorburn, G.D. (1978a) Induction of premature parturition in sheep: adrenocorticotrophin and corticosteroid changes during infusion of synacthen into the foetus. *Acta Endocrinologica*, **87**, 192–202.

Jones, C.T., Kendall, J.Z., Ritchie, J.W.K., Robinson, J.S. and Thorburn, G.D. (1978b) Adrenocorticotrophin and corticosteroid changes during dexamethasone infusion to intact and synacthen infusion to hypophysectomized foetuses. *Acta Endocrinologica*, **87**, 203–211.

Kendall, J.Z. Challis, J.R.G., Hart, I.C., Jones, C.T., Mitchell, M.D., Ritchie, J.W.K., Robinson, J.S. and Thorburn, G.D. (1977) Steroid and prostaglandin concentrations in the plasma of pregnant ewes during infusion of adrenocorticotrophin or dexamethasone to intact or hypophysectomized fetuses. *Journal of Endocrinology*, **75**, 59–71.

Kennedy, P.C. (1971) Interaction of fetal disease and the onset of labour in cattle and sheep. *Federation Proceedings*, **30**, 110–113.

Kennedy, P.C., Kendrick, J.W. and Stormont, C. (1957) Adenohypophyseal aplasia, an inherited defect associated with abnormal gestation in guernsey cattle. *Cornell Veterinarian*, **47**, 160–178.

Kojima, I., Kojima, K. and Rasmussen, H. (1985) Role of calcium and cAMP in the action of adrenocorticotropin on aldosterone secretion. *Journal of Biological Chemistry*, **260**, 4248–4256.

Laychock, S.G. and Rubin, R.P. (1975) ACTH-induced prostaglandin biosynthesis from 3H-arachidonic acid by adrenocortical cells. *Prostaglandins*, **10**, 529–540.

Lefkowitz, R.J., Roth, J. and Pastan, I. (1971) ACTH-receptor interaction in the adrenal: a model for the initial step in the action of hormones that stimulate adenyl cyclase. *Annals of the New York Academy of Sciences*, **185**, 195–209.

Levidiotis, M., Oldfield, B. and Wintour, E.M. (1987) Corticotrophin-releasing factor and arginine vasopressin fibre projections to the median eminence of fetal sheep. *Neuroendocrinology*, **46**, 453–456.

Li, Z.G., Park, D. and La Bella, F.S. (1989) Adrenocorticotropin $_{1-10}$ and $_{11-24}$ promote adrenal steroidogenesis by different mechanisms. *Endocrinology*, **125**, 592–596.

Liggins, G.C. (1968) Premature parturition after infusion of corticotrophin or cortisol into fetal lambs. *Journal of Endocrinology*, **42**, 323–325.

Liggins, G.C. (1969a) The foetal role in the initiation of parturition in the ewe, in, *Foetal Autonomy*, (eds. G.E.W. Wolstenholme and M. O'Connor) Ciba Foundation Symposium, Churchill, London pp. 218–244.

Liggins, G.C. (1969b) Premature delivery of foetal lambs infused with glucocorticoids. *Journal of Endocrinology*, **45**, 515–523.

Liggins, G.C. and Kennedy, P.C. (1968) Effects of electrocoagulation of the foetal lamb hypophysis on growth and development. *Journal of Endocrinology*, **40**, 371–381.

Liggins, G.C., Kennedy, P.C. and Holm, L.W.

(1967) Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. *American Journal of Obstetrics and Gynecology*, **98**, 1080–1086.

Liggins, G.C., Fairclough, R.J., Grieves, S.A., Forster, C.S. and Knox, B.S. (1977) Parturition in the sheep, in, *The Fetus and Birth*, (eds J. Knight and M. O'Connor), Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 5–30.

Liggins, G.C., Kitterman, J.A. and Forster, L.S. (1979) Fetal maturation related to parturition. *Animal Reproduction Science*, **2**, 193–207.

Liggins, G.C., Scroop, G.C. and Haughey, K.G. (1982) Comparison of the effects of prostaglandin E_2, prostacyclin and 1–24 adrenocorticotrophin on plasma cortisol levels of fetal sheep. *Journal of Endocrinology*, **95**, 153–162.

Lis, M., Hamet, P., Gutkowska, J., Maruice, G., Seidah, N.G., Lariviére, N., Chrétien, M. and Genest, J. (1981) Effect of *N*-terminal portion of pro-opiomelanocortin on aldosterone release by human adrenal adenoma *in vitro*. *Journal of Clinical Endocrinology and Metabolism*, **52**, 1053–1056.

Louis, T.M., Challis, J.R.G., Robinson, J.S. and Thorburn, G.D. (1976) Rapid increase of foetal corticosteroids after prostaglandin E_2. *Nature*, **264**, 797–799.

Lye, S.J., Sprague, C.L., Mitchell, B.F. and Challis, J.R.G. (1983) Activation of ovine fetal adrenal function by pulsatile or continuous administration of $ACTH_{1-24}$. I. Effects on fetal plasma corticosteroids. *Endocrinology*, **113**, 770–776.

McIlhinney, R.A.J. and Schulster, D. (1975) Studies on the binding of ^{125}I-labelled corticotrophin to isolated rat adrenocortical cells. *Journal of Endocrinology*, **64**, 175–184.

McMillen, I.C., Mercer, J.D. and Thorburn, G.D. (1988) Proopiomelanocortin messenger RNA levels in the fetal sheep pituitary fall before birth. *Journal of Molecular Endocrinology*, **1**, 141–145.

McMillen, I.C., Antolovich, G.C., Mercer, J.D., Perry, R.A. and Silver, M. (1990) Pro-opiomelanocortin messenger RNA levels are increased in the anterior pituitary of the sheep fetus after adrenalectomy in late gestation. *Neuroendocrinology*, **52**, 297–302.

Madill, D. and Bassett, J.M. (1973) Corticosteroid release by adrenal tissue from foetal and newborn lambs in response to corticotrophin stimulation in a perifusion system *in vitro*. *Journal of Endocrinology*, **58**, 75–87.

Magyar, D.M., Fridshal, D., Elsner, C.W., Glatz, T., Eliot, J. Klein, A.H., Lowe, K.C., Buster, J.E. and Nathanielsz, P.W. (1980a) Time-trend analysis of plasma cortisol concentrations in the fetal sheep in relation to parturition. *Endocrinology*, **107**, 155–159.

Magyar, D.M., Devaskar, J., Fridshal, D., Buster, J.E. and Nathanielsz, P.W. (1980b) Responsiveness and maximum secretory capacity of isolated fetal lamb adrenocortical cells throughout the last third of gestation. *Endocrinology*, **107**, 1582–1586.

Malet, C., Scherrer, H., Saavedra, J.M. and Dray, F. (1982) Specific binding of ^{3}H- prostaglandin E_2 to rat brain membranes and synaptosomes. *Brain Research*, **236**, 227–233.

Malpas, P. (1933) Postmaturity and malformations of the fetus. *Journal of Obstetrics and Gynaecology of the British Empire*, **40**, 1046–1053.

Manchester, E.L. and Challis, J.R.G (1982) The effects of adrenocorticotropin, guanylylimidodiphosphate, dibutyrl adenosine 3′,5′-monophosphate and exogenous substrates on corticosteroid output by ovine fetal adrenal cells at different times in pregnancy. *Endocrinology*, **111**, 889–895.

Mason, J.I., France, J.T., Magness, R.R., Murry, B.A. and Rosenfeld, C.R. (1989) Ovine placental steroid 17α-hydroxylase/C-17,20-lyase, aromatase and sulphatase in dexamethasone-induced and natural parturition. *Journal of Endocrinology*, **122**, 351–359.

Mendelson, C.R. (1988) Mechanisms of hormone action, in, *Textbook of Endocrine Physiology*, (eds J.E. Griffin and S.R. Ojeda) Oxford University Press, New York, pp. 28–55.

Mulvogue, H.M., McMillen, I.C., Robinson, P.M. and Perry, R.A. (1986) Immunocytochemical localisation of pro-γMSH, γMSH, ACTH and β-endorphin/β-lipotrophin in the fetal sheep pituitary: an ontogenetic study. *Journal of Developmental Physiology*, **8**, 355–368.

Nathanielsz, P.W., Jack, P.M.B., Krane, E., Thomas, A.L., Ratter, S. and Rees, L.H. (1977) The role and regulation of corticotropin in the fetal sheep, in, *The Fetus and Birth*, (eds M. O'Connor and J. Knight), Ciba Foundation Symposium No. 47, Elsevier, Amsterdam, pp. 73–91.

Nathanielsz, P.W., Elsner, C., Magyar, D., Fridshal, D., Freeman, A. and Buster, J.E. (1982)

Time trend analysis of plasma unconjugated and sulfoconjugated estrone and Δ^5-steroids in fetal and maternal sheep plasma in relation to spontaneous parturition at term. *Endocrinology*, **110**, 1402–1407.

Norman, L.J. and Challis, J.R.G. (1987a) Synergism between systemic CRF and AVP on ACTH release *in vivo* varies as a function of gestational age in the ovine fetus. *Endocrinology*, **120**, 1052–1058.

Norman, L.J. and Challis, J.R.G. (1987b) Dexamethasone inhibits ovine corticotropin- releasing factor (oCRF), arginine vasopressin (AVP), and oCRF and AVP stimulated release of ACTH during the last third of pregnancy in the sheep fetus. *Canadian Journal of Physiology and Pharmacology*, **65**, 1186–1192.

Norman, L.J., Lye, S.J., Wlodek, M.E. and Challis, J.R.G. (1985) Changes in pituitary responses to synthetic ovine corticotrophin releasing factor in fetal sheep. *Canadian Journal of Physiology and Pharmacology*, **63**, 1398–1403.

Ojeda, S.R., Naor, Z. and McCann, S.M. (1978) Prostaglandin E levels in hypothalamus, median eminence and anterior pituitary of rats of both sexes. *Brain Research*, **149**, 274–277.

Oka, K., Lund, J. and Mason, J.I. (1989) Prostaglandin-promoted induction of fetal bovine adrenal steroid 17α-hydroxylase (abstract). *Society for Gynaecologic Investigation*, San Diego.

Ozolins, I.Z., Young, I.R. and McMillen I.C. (1990) Effect of cortisol infusion on basal and corticotropin-releasing factor (CRF)-stimulated plasma ACTH concentrations in the sheep fetus after surgical isolation of the pituitary. *Endocrinology*, **127**, 1833–1840.

Parkes, M.J., Linton, E.A., Moore, P.J., Hanson, M.A. and Lowry, P.J. (1988) Levels of ACTH (measured by two site immunoradiometric assay) and cortisol in chronically sampled fetal sheep during late pregnancy and parturition. *The Liggins Symposium on Fetal Physiology and Medicine*, Rotorua, New Zealand, p. 57.

Pedersen, R.C. and Brownie, A.C. (1980) Adrenocortical response to corticotropin is potentiated by part of the amino-terminal region of pro-corticotropin/endorphin. *Proceedings of the National Academy of Sciences of the USA*, **77**, 2239–2243.

Peng, T.C., Six, K.M. and Munson, P.L. (1970) Effects of prostaglandin E_1 on the hypothalamo-hypophyseal-adrenocortical axis in rats. *Endocrinology*, **86**, 202–206.

Perry, R.A., Mulvogue, H.M., McMillen, I.C. and Robinson, P.M. (1985) Immunohistochemical localization of ACTH in the adult and fetal sheep pituitary. *Journal of Developmental Physiology*, **7**, 397–494.

Petraglia, F., Sawchenko, P.E., Rivier, J. and Vale, W. (1987) Evidence for local stimulation of ACTH secretion by corticotrophin-releasing factor in human placenta. *Nature*, **328**, 717–719.

Pilote, S., Vallerand, P. and Borgeat, P. (1982) Transformation of arachidonic acid in the rat anterior pituitary. *Biochemical and Biophysical Research Communications*, **104**, 867–873.

Pradier, P., Dalle, M., Davicco, M.J., Lefaivre, J., Barlet, J.P. and Delost, P. (1985) Plasma ACTH, cortisol and aldosterone concentrations in chronically cannulated ovine fetuses and in lambs injected with ovine corticotropin releasing factor. *Journal of Developmental Physiology*, **7**, 259–268.

Pradier, P., Dalle, M., Tournaire, C. and Delost, P. (1988) Plasma concentrations of adrenocorticotropin-related peptides after corticotropin-releasing hormone and vasopressin injections in sheep. *Acta Endocrinologica*, **119**, 391–396.

Rainey, W.E., Oka, K., Magness, R.R. and Mason, J.I. (1991) Ovine fetal adrenal synthesis of cortisol: regulation by angiotensin II (A-II) and transforming growth factor beta (TGF-β). *Society for Gynecologic Investigation*, 38th Annual Meeting, San Antonio, Texas, Abstract 338.

Rasmussen, H., Kojima, I., Apfeldorf, W. and Barrett, P. (1986) Cellular mechanism of hormone action in the kidney: messenger function of calcium and cyclic AMP. *Kidney International*, **29**, 90–97.

Rees, L.H., Jack, P.M.B., Thomas, A.L. and Nathanielsz, P.W. (1975) Role of foetal adrenocorticotrophin during parturition in sheep. *Nature*, **253**, 274–275.

Rice, G.E. and Thorburn, G.D. (1988) The ontogeny of prostaglandin synthetic capacity (abstract). *The Liggins Symposium on Fetal Physiology and Medicine*, Rotorua, New Zealand.

Rice, G.E., Wong, M.H. and Thorburn, G.D. (1988) Gestational changes in prostaglandin synthase activity of ovine cotyledonary microsomes. *Journal of Endocrinology*, **118**, 265–270.

Risbridger, G.P., Leach Harper, C.M., Wong, M.H. and Thorburn, G.D. (1985) Gestational

changes in prostaglandin production by ovine fetal trophoblast cells. *Placenta*, **6**, 117–126.

Roebuck, M.M., Jones, C.T., Holland, D. and Silman, R. (1980) *In vitro* effects of high molecular weight forms of ACTH on the fetal sheep adrenal. *Nature*, **284**, 616–618.

Rose, J.C., Macdonald, A.A., Heymann, M.A. and Rudolph, A.M. (1978) Developmental aspects of the pituitary–adrenal axis response to hemorrhagic stress in lamb fetuses *in utero*. *Journal of Clinical Investigation*, **61**, 424–432.

Rose, J.C., Meis, P.J. and Morris, M. (1981) Ontogeny of endocrine (ACTH, vasopressin, cortisol) responses to hypotension in lamb fetuses. *American Journal of Physiology*, **240**, E656–E661.

Rose, J.C., Meis, P.J., Urban, R.B. and Greiss, F.C. (1982) *In vivo* evidence for increased adrenal sensitivity to adrenocorticotropin$_{(1-24)}$ in the lamb fetus late in gestation. *Endocrinology*, **111**, 80–85.

Sarutz, T. and Kaplan, N.M. (1972) Adrenocortical steroidogenesis: the effects of prostaglandins. *Journal of Clinical Investigation*, **51**, 2246–2251.

Schwartz, J. (1990) Evidence for intrapituitary intercellular control of adrenocorticotropin secretion. *Molecular and Cellular Endocrinology*, **68**, 77–83.

Schwartz, J. and Vale, W. (1988) Dissociation of the adrenocorticotropin-secretory responses to corticotropin-releasing factor (CRF) and to vasopressin or oxytocin by using a specific cytotoxic analog of CRF. *Endocrinology*, **122**, 1695–1701.

Schwartz, J., Pham, T., Rao, A. and Funder, J.W. (1991) Effect of AVP on susceptibility of ovine pituitary cells to a cytotoxic analog of CRF. *American Journal of Physiology*, **260**, E905–E909.

Silman, R.E., Holland, D., Chard, T., Lowry, P.J., Hope, J., Rees, L.H., Thomas, A. and Nathanielsz, P. (1979) Adrenocorticotrophin-related peptides in adult and fetal sheep pituitary glands. *Journal of Endocrinology*, **81**, 19–34.

Spät, A., Siklós, P., Antoni, F.A., Nagy, K. and Szirányi, K. (1977) Effect of prostaglandin synthetase inhibitors of basal and ACTH-stimulated steroid synthesis by separated adrenocortical zones. *Journal of Steroid Biochemistry*, **8**, 293–298.

Spiegelberg (1882) *Textbook of Midwifery*.

Strott, C.A., Sundel, H. and Stahlman, M.T. (1974) Maternal and fetal plasma progesterone, cortisol, testosterone and 17β-estradiol in preparturient sheep: response to fetal ACTH infusion. *Endocrinology*, **95**, 1327–1339.

Thomas, S.J., Wilson, D.W., Pierrepoint, C.G., Cameron, E.H.D. and Griffiths, K. (1976) Measurement of cortisol, cortisone, 11-deoxycortisol and corticosterone in fetal sheep plasma during the perinatal period. *Journal of Endocrinology*, **68**, 181–189.

Thompson, M.E. and Hedge, G.A. (1978) Inhibition of corticotropin secretion by hypothalamic administration of indomethacin. *Neuroendocrinology*, **25**, 212–220.

Thompson, M.E. and Hedge, G.A. (1981) Corticosterone secretion after inhibition of prostaglandin synthesis by indomethacin. *American Journal of Physiology*, **240**, E131–E135.

Thorburn, G.D. (1987) The orchestration of parturition: does the fetus play the tune?, in, *Endocrinology and Physiology of Reproduction*, (eds P.C.K. Leung, D.T. Armstrong, K.B. Ruf, W.H. Moger and H.G. Friesen), Plenum Publishing, New York, pp. 331–353.

Thorburn, G.D. (1991) The placenta, prostaglandins and parturition: a review. *Reproduction, Fertility and Development*, **3**, 277–294.

Thorburn, G.D. and Rice, G.E. (1990) Placental PGE_2 and the initiation of parturition in sheep, in, *Eicosanoids in Reproduction*, (ed. M.D. Mitchell), CRC Press, Boca Raton, Florida, pp. 73–86.

Thorburn, G.D., Hooper, S.B., Rice, G.E. and Fowden, A.L. (1988) Luteal regression and parturition: a comparison, in, *Cellular and Integrative Mechanisms in the Onset of Labor – What are the Research Priorities?* (eds D. McNellis, J.R.G. Challis, P. MacDonald, P. Nathanielsz and J. Roberts) NICHD Workshop, Bethesda, Perinatology Press, New York, pp. 185–210.

Thorburn, G.D., Deayton, J.M., Young, I.R., and Ralph, M.M. (1989) The effects of early fetal hypophysectomy on ovine placental PGE_2 and steroid biosynthesis. *Journal of Reproduction and Fertility*, Abstract Series No. 3 Abstract 49.

Thorburn, G.D., Hollingworth, S.A. and Hooper, S.B. (1991) The trigger for parturition in sheep: fetal hypothalamus or placenta? *Journal of Developmental Physiology*, **15**, 71–79.

Vale, W., Rivier, C., Yang, L., Minick, S. and Guillemin, R. (1978) Effects of purified hypothalamic corticotropin releasing factor and other substances on the secretion of ACTH and β-endorphin-like immunoreactivities *in vitro*. *Endocrinology*, **103**, 1910–1915.

van Rensburg, S.J. (1971) Reproductive physiology and endocrinology of normal and habitu-

ally aborting angora goats. *Onderstepoort Journal of Veterinary Research*, **38**, 1–62.

Vilhardt, H. and Hedqvist, P. (1970) A possible role of prostaglandin E_2 in the regulation of vasopressin secretion in rats. *Life Sciences*, **9**, 825–830.

Vlaskovska, M. and Knepel, W. (1984) Beta-endorphin and adrenocorticotropin release from rat adenohypophysis *in vitro*: evidence for local modulation by arachidonic acid metabolites of the cyclooxygenase and lipoxygenase pathway. *Neuroendocrinology* **39**, 334–342.

Vlaskovska, M., Hertting, G. and Knepel, W. (1984) Adrenocorticotropin and beta-endorphin release from rat adenohypophysis *in vitro*: inhibition by prostaglandin E_2 formed locally in response to vasopressin and corticotropin-releasing factor. *Endocrinology*, **115**, 895–903.

Wango, E., Wooding, F.B.P. and Heap, R.B. (1988) Differences in the role for prostaglandin E_2 in steroid synthesis of isolated binucleate cells from sheep and goat placenta (abstract). *Journal of Endocrinology*, Suppl, 117.

Warner, W. and Rubin, R.P. (1975) Evidence for a possible prostaglandin link in ACTH-induced steroidogenesis. *Prostaglandins*, **9**, 83–95.

Weidenfeld, J., Siegel, R.A., Conforti, N. and Chowers, I. (1980) Effects of systemically administered indomethacin on basal and stress-induced ACTH and corticosterone secretion in the male rat. *Neuroendocrinology*, **31**, 81–84.

Weidenfeld, J., Siegel, R.A., Conforti, N., Feldman, S. and Chowers, I. (1981) Site and mode of action of indomethacin on the hypothalamo-hypophyseal-adrenal axis: a temporal study in intact, hypothalamic-deafferentated, and hypothalamic-lesioned male rats. *Endocrinology*, **109**, 205–209.

White, A., Smith, H., Hoadley, M., Dobson, S.H. and Ratcliff, J.G. (1987) Clinical evaluation of a two-site immunoradiometric assay for adrenocorticotropin in unextracted human plasma using monoclonal antibodies. *Clinical Endocrinology*, **26**, 41–52.

de Wied, D., Witter, A., Versteeg, D.H.G. and Mulder, A.H. (1969) Release of ACTH by substances of central nervous system origin. *Endocrinology*, **85**, 561–569.

Wintour, E.M., Brown, E.H., Denton, D.A., Hardy, K.J. McDougall, J.G., Oddie, C.J. and Whipp, G.T. (1975) The ontogeny and regulation of corticosteroid secretion by the ovine foetal adrenal. *Acta Endocrinologica* **79**, 301–316.

Wintour, E.M., Brown, E.H. and Denton, D.A. (1977) *In vitro* and *in vivo* adrenal cortisol steroid production by fetal sheep: effect of angiotensin II, sodium deficiency, ACTH. *Research on Steroids*, **3**, 475–485.

Wintour, E.M., Coghlan, J.P., Hardy, K.J., Hennessy, D.P., Lingwood, B.E. and Scoggins, B.A. (1980) Adrenal corticosteroids and immunoreactive ACTH in chronically cannulated ovine fetuses with bilateral adrenalectomy. *Acta Endocrinologica*, **95**, 546–552.

Wood, C.E. (1986) Sensitivity of cortisol-induced inhibition of ACTH and renin in fetal sheep. *American Journal of Physiology*, **250**, R795–R802.

Wood, C.E. (1987) Does a decrease in cortisol negative feedback efficacy precede ovine parturition? *American Journal of Physiology*, **252**, R624–R627.

Wood, C.E. (1988) Insensitivity of near-term fetal sheep to cortisol: possible relation to the control of parturition. *Endocrinology*, **122**, 1565–1572.

Yamamoto, M., Share, L. and Shade, R.E. (1976) Vasopressin release during ventriculo-cisternal perfusion with prostaglandin E_2 in the dog. *Journal of Endocrinology* **71**, 325–331.

Yanagibashi, K. (1979) Calcium ion as 'second messenger' in corticoidogenic action of ACTH. *Endocrinology (Japan)*, **26**, 227–232.

Yang, K., Challis, J.R.G., Han, V.K.M. and Hammond, G.L. (1991) Proopiomelanocortin mRNA levels increase in the fetal sheep pituitary during the last week of gestation. *Society for Gynecologic Investigation*, (abstract), 38th Annual Meeting, San Antonia, Texas, p.219.

REGULATION OF POST-PARTUM FERTILITY IN LACTATING MAMMALS

11

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11.1 INTRODUCTION

During lactation, the suckling offspring controls three major components of lactation: the production of milk within the mammary gland, the ejection of this milk to the offspring and, in many species, the suppression of ovarian activity or delay in implantation. Delaying a new pregnancy ensures sufficient time before the birth of the next offspring to permit adequate growth of the current offspring during lactation. This chapter will review endocrine changes in lactation and their relationship to fertility regulation post partum.

11.2 ENDOCRINE CHANGES ASSOCIATED WITH MILK PRODUCTION

The onset of milk secretion at parturition, lactogenesis, occurs in all mammals and appears to be related not to an increase in the secretion of lactogenic hormones but to a removal of an inhibition of their action at the level of the mammary gland (Forsyth, 1986). There is considerable species variation in prolactin concentrations during pregnancy, but in all species for which data are available prolactin shows a large periparturient increase. If prolactin is suppressed around parturition by the dopamine agonist, bromocriptine, the normal onset of lactogenesis is blocked in rats (Bohnet *et al.*, 1977), rabbits (Djiane and Durand, 1977), pigs (Taverne *et al.*, 1982), monkeys (Schallenberger *et al.*, 1981) and human (Cooke *et al.*, 1976; McNeilly, 1977; F. Peters *et al.*, 1986), confirming the essential role of prolactin. In contrast, suppression of prolactin at parturition in ruminants delays rather than prevents lactogenesis if milking is continued (Akers *et al.*, 1981; A.J. Davis *et al.*, 1983; Forsyth and Lee, 1993), although milk yield may be reduced by below average peripartum prolactin at a later stage of lactation in cows (Erb *et al.*, 1980), but not goats (Forsyth and Lee, 1993).

It appears, therefore, that in the majority of species, while prolactin is essential for the initiation of milk production at parturition, it is triggered by a withdrawal of progesterone (Kuhn, 1983) as a consequence of cessation of luteal function or that delivery of the placenta after term is a major triggering factor (see Cowie *et al.*, 1980; Forsyth, 1986). Jahn *et al.* (1989) show progesterone inhibition of casein synthesis *in vitro* in rabbit mammary explants by using physiological concentrations of prolactin and cortisol. Removal of prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), an active inhibitor of milk secretion, may also be involved. In late pregnant goats the mammary gland synthesizes $PGF_{2\alpha}$. Its secretion is switched from blood to milk 3–5 days prepartum (Maule Walker, 1984).

Once lactation is established, the continuation of milk production in all species is dependent on the pituitary gland (Cowie *et al.*, 1980). Experiments using hormone replacement in hypophysectomized lactating animals and *in vitro* organ culture of mammary tissue have shown a requirement for prolactin, growth hormone, insulin, thyroid hormones and glucocorticoids, the proportions and relative importance of which varies with species (Cowie *et al.*, 1980). In the following sections the change in secretion of these various hormones and their relative importance will be considered.

11.2.1 PROLACTIN

In all species so far studied, prolactin levels are raised during lactation (rat, Amenomori *et al.*, 1970; Terkel *et al.*, 1972; mice, Sinha *et al.*, 1974; Markoff *et al.*, 1981; rabbit, A.S. McNeilly and Friesen, 1978; Fuchs *et al.*, 1984; Californian ground squirrel, Thordason *et al.*, 1987; dog, Concannon *et al.*, 1978; blue fox, Mondain-Monval *et al.*, 1985; tammar wallaby, Hinds and Tyndale-Biscoe, 1982, 1985; and Bennett's wallaby, Curlewis *et al.*, 1986; bush-tailed possum, Hinds and Janssens, 1986; eastern quoll, Hinds and Merchant,

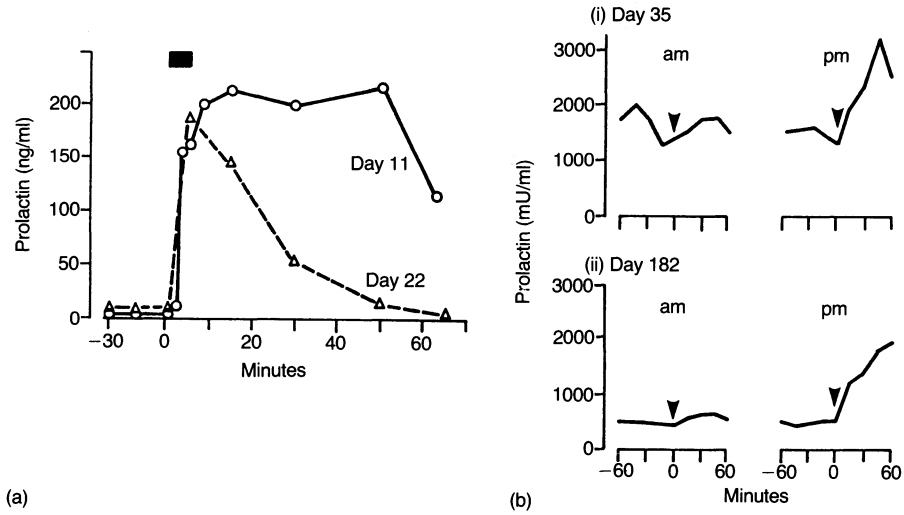

Figure 11.1 Effect of time post-partum on the suckling-induced release of prolactin in the rabbit (A) and human (B). The amount of milk taken and the duration of suckling was similar at the different stages of lactation but the amount of prolactin decreased. (From A.S. McNeilly and Friesen, 1978.)

1986; pig, Bevers *et al.*, 1978, 1981; van Landeghem and van de Wiel, 1978; Stevenson *et al.*, 1981; goat, Hart, 1974; sheep, Kann and Denamur, 1974; Lamming *et al.*, 1974; cow, Koprowski and Tucker, 1973a,b; red deer, Loudon *et al.*, 1983; mare, Worthy *et al.*, 1986; Weist and Thompson, 1987; elephant, A.S. McNeilly *et al.*, 1983a; rhesus monkey, Schallenberger *et al.*, 1981; marmoset monkey: A.S. McNeilly *et al.*, 1981; human, e.g. Delvoye *et al.*, 1978; Duchen and McNeilly, 1980; Howie *et al.*, 1980; A.S. McNeilly, 1980).

Prolactin is released in response to the suckling stimulus (Figures 11.1 to 11.3), and there is no evidence that prolactin levels increase in response to exteroceptive or other stimuli related to suckling or milking other than direct teat or nipple stimulation (Figures 11.2 and 11.3; rat, Voloschin and Tramezzani, 1984; Mattheij and Swarts, 1987; rabbit, A.S. McNeilly and Friesen, 1978; Fuchs *et al.*, 1984; goat, Hart and Linzell, 1977; human, Noel *et al.*, 1974; McNeilly *et al.*, 1983b).

In most eutherian species the prolactin response to suckling is greatest during early to mid-lactation and declines as lactation progresses (Figure 11.1; rat, Amenomori *et al.*, 1970; rabbit, A.S. McNeilly and Friesen, 1978; Fuchs *et al.*, 1984; sow, van Landegham and van der Wiel, 1978; sheep, J.R. McNeilly, 1972; goat, Hart, 1974; cow, Koprowski and Tucker, 1973a; Whisnant *et al.*, 1986a; human, Glasier *et al.*, 1984a; Johnston and Amico, 1986). However, the wide variation in suckling pattern and nursing behaviour between species results in a variable pattern of prolactin during lactation. In rabbits, which suckle only once per day for 2–3 min, prolactin increases immediately suckling begins and prolactin levels remain elevated for up to 4 h after the end of suckling (Figure 11.1; A.S. McNeilly and Friesen, 1978; Fuchs *et al.*, 1984). In the rat, sheep, cow, goat, rhesus monkey and human, prolactin also increases at the start of suckling but tends to decline at the end of the suckling period (Figures 11.2 and 11.3; Frantz *et al.*, 1972; Lamming *et al.*, 1974; Kann and Denamur, 1974; Noel *et al.*, 1974; Hart, 1975a; Frawley *et al.*, 1983; Glasier *et al.*, 1984a; Grosvenor *et al.*, 1986; Johnston and Amico, 1986), although this pattern may

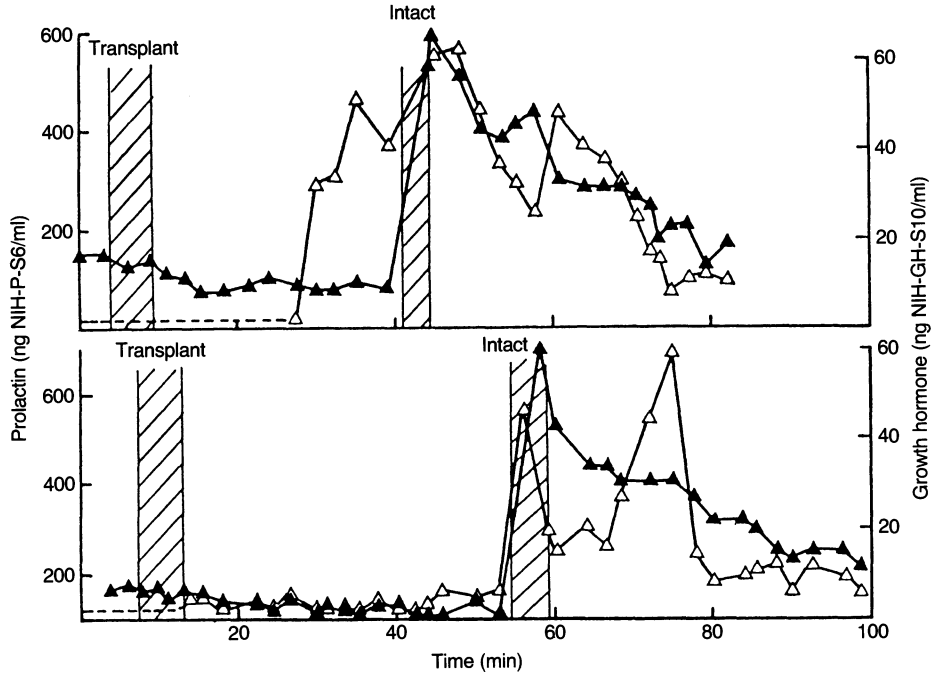

Figure 11.2 Changes in the plasma levels of prolactin (▲) and growth hormone (before, during and after milking (△) denervated, transplanted and intact innervated mammary glands in three goats. Note that prolactin concentrations increase only after milking of the innervated intact udder while, growth hormone release occurs after milking both the denervated and innervated udders. (From Hart and Linzell, 1977.)

vary within species throughout lactation (e.g. human, Hennart *et al.*, 1981; Glasier *et al.*, 1984a).

Studies of the mechanisms controlling the release of prolactin in response to the suckling stimulus have often utilized, as a model, the lactating rat deprived of all but one of its litter for a variable period before the pups are reattached. An immediate increase in prolactin is then seen at the start of suckling (e.g. Isherwood and Cross, 1980; Sirinathsinghji and Martini, 1984; Grosvenor *et al.*, 1986; Neill, 1986). However, this response is not normal in the rat, which spends up to 18 h per day nursing. Studies in which the changes in prolactin and suckling behaviour have been monitored throughout the day show considerable variation in suckling activity during each 24-h period and no precise relationship between suckling and prolactin levels (van der Schoot *et al.*, 1978; Sodersten and Eneroth, 1984; Nagy *et al.*, 1985, 1986a), a pattern also seen in the sow (Mattioli *et al.*, 1986). Indeed in the rat, maximum levels of prolactin could occur at times when mothers were away from the pups (Sodersten and Eneroth, 1984). In the rhesus monkey (Frawley *et al.*, 1983; Aso and Williams, 1985) and human (Tyson *et al.*, 1972; Noel *et al.*, 1974; Glasier *et al.*, 1984a) when breast feeds are infrequent with an interval of 4–5 h, a clear increase in prolactin is seen at each suckling period. However, when the frequency of feeds increases, the prolactin response to each feed diminishes but the overall basal level of prolactin is significantly increased (Delvoye *et al.*, 1977; Madden *et al.*, 1978; Stern *et al.*, 1986). Thus,

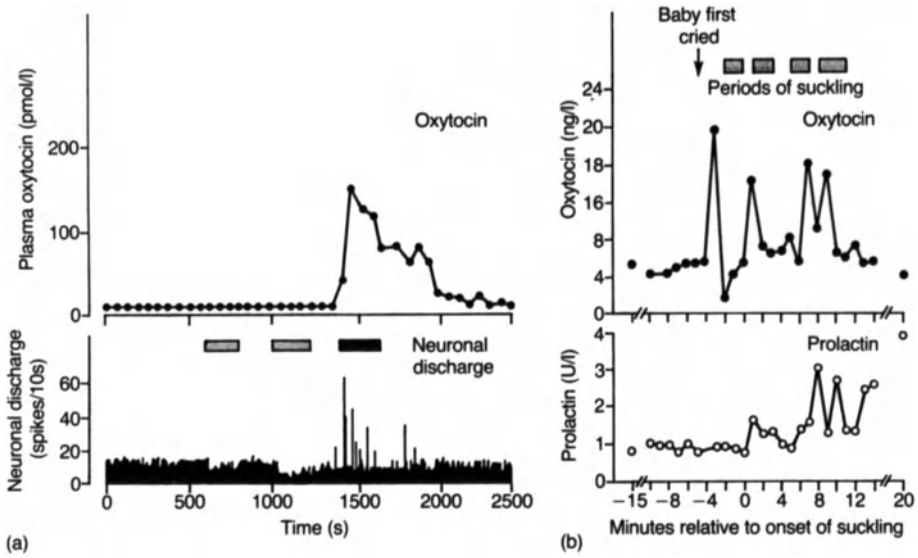

Figure 11.3 Changes in plasma levels of oxytocin in association with nursing in (A) a rabbit and (B) a human. In the rabbit oxytocin neurone activity is slow and irregular before nursing and plasma levels of oxytocin are undetectable. While the sight of the pups (▨) before nursing did not alter neuronal discharge or plasma oxytocin, the onset of nursing (■) was associated with an increase in the rate of firing of the oxytocin neurone followed by a sustained rise in plasma levels of oxytocin. In contrast, in the human, a large pulsatile release of oxytocin occurred when the baby cried before the onset of nursing. Thereafter, plasma levels of oxytocin increased in a pulsatile manner during the period of nursing. Plasma levels of prolactin only increased after the onset of suckling. (Redrawn from A.S. McNeilly *et al.*, 1983b, and Summerlee *et al.*, 1986.)

the frequency of suckling is associated with an overall increase in prolactin levels and a reduction in the amount of prolactin released at each suckling episode.

The strength of the suckling stimulus may also alter the amount of prolactin released at each suckling episode. More prolactin may be released in response to suckling of hungry pups in the rat (Grosvenor *et al.*, 1986; Jakubowski and Terkel, 1986a,b), and at milking if two teats rather than one are stimulated in the goat (Hart and Linzell, 1977). Basal levels of prolactin are greater in underfed red deer, whose calves suckle more frequently (Loudon *et al.*, 1983), but apparently not in the cow (Wheeler *et al.*, 1982), and are higher in malnourished humans throughout lactation (P.G. Lunn *et al.*, 1980; Hennart *et al.*, 1985) and in lactating marmoset monkeys suckling twins rather than single offspring (A.S. McNeilly *et al.*, 1981).

Plasma prolactin levels are only marginally elevated during the early stages of lactation when the young is very small in the bush-tailed possum (Hinds and Janssens, 1986), eastern quoll (Hinds and Merchant, 1986) and tammar (Hinds and Tyndale-Biscoe, 1982, 1985) and Bennett's wallaby (Curlewis *et al.*, 1986). When the joey begins its growth spurt in mid-lactation, prolactin levels increase dramatically, presumably in response to a greatly increased suckling activity and demand for milk in all these marsupials, although there may be some confounding effects of season (Tyndale-Biscoe and Hinds, 1984). Prolactin levels decline only when the joey leaves the pouch, returning to suckle only infrequently. In tammar wallabies, an

increase in prolactin receptors in the suckled gland in very early lactation probably enables this gland to respond selectively to low circulating prolactin (F. Stewart, 1984). The change in the pattern of prolactin in response to suckling or milking in goats, sheep and cattle is similarly confounded to some extent by the seasonal increase in prolactin which occurs in all three species with increasing day length (sheep, Walton *et al.*, 1977; goat, Hart, 1975b; cow, Peters and Tucker, 1978; Webb and Lamming, 1981). Since young ruminants are normally born in the springtime, this coincides with the time of the natural seasonal increase in prolactin. Nevertheless, prolactin levels in lactating ewes, whether in the breeding season (Clarke *et al.*, 1984) or anoestrus (Kann and Denamur, 1974; Lamming *et al.*, 1974), are greater than in non-lactating seasonally anoestrous ewes, and the prolactin response to suckling in both the sheep (J.R. McNeilly, 1972) and goat (Hart, 1975a) declines throughout lactation. The prolactin levels are similar in suckling and milked cows (Carruthers and Hafs, 1980; J.F. Smith *et al.*, 1981). In goats, milk yield increase resulting from more frequent milking is ultimately associated with an increase in prolactin receptors, an effect local to the more frequently milked gland within an animal (McKinnon *et al.*, 1988).

The control of prolactin secretion is complex and the mechanisms underlying the increased release of prolactin during suckling and throughout lactation remain poorly understood. The main control over prolactin is provided by tonic dopaminergic inhibition from the hypothalamus, with ovarian oestradiol providing both chronic and acute endocrine stimulation. A large number of prolactin secretagogues have been identified, but their physiological relevance remains uncertain. There is now evidence that prolactin secretion is subject also to paracrine control from the intermediate lobe and to controls operating within the anterior pituitary (Ben-Jonathan and Liu, 1992).

The primary prolactin-inhibiting factor is dopamine (Ben-Jonathan 1985). Tuberoinfundibular (TIDA) neurones terminate in the median eminence and release dopamine to reach the anterior pituitary via the long portal vessels. Tuberohypophysial (THDA) neurones project separately to the neural and the intermediate lobes of the posterior pituitary. They supply a lesser dopamine input to the anterior pituitary from the neural lobe via the short portal vessels. In the intermediate lobe, dopaminergic terminals synapse with glandular cells. Evidence in the rat suggests that the acute suckling stimulus causes a transient decline in dopamine levels in the hypophysial portal vessels (Plotsky and Neill, 1982) and the pituitary (Duker and Wuttke, 1985), allowing the prolactin in the pituitary to be transformed into a readily releasable form (Grosvenor *et al.*, 1986), which is then secreted into the circulation. A chronic reduction in the amount of dopamine released from TIDA neurones of the hypothalamus into the portal vessels may contribute to the maintenance of high prolactin levels throughout lactation, since hypothalamic dopamine turnover is reduced in lactation (Selmanoff and Wise, 1981; Demarest *et al.*, 1983) and the ability of prolactin to stimulate dopamine release from these neurones is also reduced (short-loop feedback, Demarest *et al.*, 1983).

In lactating rats, removal of the posterior pituitary elevates basal prolactin release, confirming an involvement of THDA neurones in tonic prolactin inhibition. However, suckling fails to increase prolactin in posterior lobectomized rats (Ben-Jonathan, 1985; Murai and Ben-Jonathan, 1987). Prolactin-releasing factor activity has now been identified in the posterior pituitaries of many species (fish, mouse, rat, hamster, rabbit, pigs, cows, sheep, monkeys and humans, Thomas *et al.*, 1989; Mori *et al.*, 1990; Averill *et al.*, 1991; Ben-Jonathan *et al.*, 1991; Ellerkmann *et al.*, 1991), but its exact nature remains to be determined. In the rat, the activity is localized

mainly in the intermediate lobe (Laudon *et al.*, 1990). Recently, Ellerkmann *et al.* (1992) have found in an *in vitro* model that *N*-acetylated α-melanocyte-stimulating hormone (and/or β-endorphin) from the intermediate lobe rapidly recruits anterior pituitary cells into the prolactin-secreting pool by the removal of a post-translational block to prolactin synthesis (Porter *et al.*, 1992).

In the model proposed by Ben-Jonathan *et al.* (1991), dopamine is suggested to exert tonic inhibition over the secretion of the posterior pituitary prolactin-releasing factor, as well as prolactin itself. Suckling acts to suppress dopamine and a large amplification of the prolactin secretory response results from removal of inhibition from lactotrophs plus increased release of and response to the releasing factor. In addition, and as first postulated by Benson and Folley (1956), oxytocin released from the neural lobe by suckling could also amplify prolactin release.

In several species the release of prolactin and, by implication, the changes in dopamine turnover appear to be mediated by a suckling-induced increase in opiate tone within the hypothalamus. Thus, blockade of opioid receptors with naloxone in the rat (Sirinathsinghji and Martini, 1984; Selmanoff and Gregerson, 1986), pig (Mattioli *et al.*, 1986), sheep (Gregg *et al.*, 1986; P.G. Knight *et al.*, 1986; Malven and Hudgens, 1987) and cow (Gregg *et al.*, 1986; Whisnant *et al.*, 1986a–c) results in a decrease in prolactin levels during lactation, while systemic administration of β-endorphin (Selmanoff and Gregerson, 1986) will increase prolactin. In the ewe, suckling causes a significant increase in the concentration of β-endorphin in the hypophysial portal vessels (Gordon *et al.*, 1987), although this may not be a specific effect. While naloxone apparently does not inhibit the prolactin response to breast stimulation in lactating humans (Lodico *et al.*, 1983; Cholst *et al.*, 1984; Tay *et al.*, 1993), there seems adequate evidence to imply that suckling causes the release of prolactin by a pathway at least involving an alteration in opioid tone within the hypothalamus.

The importance of prolactin for the maintenance of milk production in lactation varies, but it appears to be essential in most species other than ruminants. Thus, the specific suppression of prolactin with bromocriptine (Bohnet *et al.*, 1977; Madon *et al.*, 1986a) or prolactin antiserum (Nagai *et al.*, 1986) in the rat, and with bromocriptine in the mouse (C.H. Knight *et al.*, 1986) rabbit (Taylor and Peaker, 1975), Bennett's wallaby (Curlewis *et al.*, 1986), dog (Jochle *et al.*, 1987), pig (Fluckiger, 1978), rhesus monkey (Schallenberger *et al.*, 1981) and women (F. Peters *et al.*, 1986) results in a cessation of milk production. This effect can be reversed by administration of exogenous prolactin with the bromocriptine in rat (Bohnet *et al.*, 1977; Madon *et al.*, 1986a) and rabbit (Taylor and Peaker, 1975). Milk production is also absent in women with an isolated prolactin deficiency (Kauppila *et al.*, 1987a; A.S. McNeilly, 1985). In women, milk production can be enhanced by a drug-induced increase in circulating prolactin concentrations during lactation (Guzman *et al.*, 1979; Kauppila *et al.*, 1981; Aono *et al.*, 1982; Ylikorkala *et al.*, 1982; Hofmeyer *et al.*, 1985). In contrast, prolactin appears to have little importance in milk production in ruminants once lactation has been established. Suppression of prolactin has little or no effect on milk yield in the cow (Karg *et al.*, 1972; V.G. Smith *et al.*, 1974) and only marginally affects milk yield in the ewe (Hooley *et al.*, 1978; Gow *et al.*, 1983). Hart (1973) reported no effect of once-daily bromocriptine on milk yield in the goat, but given three times daily it reduced milk yield by 21% (C.H. Knight *et al.*, 1990). Studies of prolactin concentrations in plasma and whey show that, as circulating prolactin declines in goats, the ratio of prolactin and in whey to plasma increases, despite an absolute decline in whey prolactin concentrations. This may indicate increased efficiency of transfer across

the gland, perhaps contributing to the maintenance of substantial milk yields (Gabai *et al.*, 1992). The mammary gland may also synthesize prolactin. Transcription of the prolactin gene has been reported in milk secretory cells of the rat mammary gland (Steinmetz *et al.*, 1993), although the substantial suppression of milk yield by bromocriptine in this species (70–100% within 2 days dependent on the stage of lactation; Flint *et al.*, 1992) suggests the contribution of locally produced prolactin to sustaining milk yield must be limited. In ruminants there is no precise correlation between prolactin levels throughout lactation and milk yield (Hart *et al.*, 1979; Kazmer *et al.*, 1986). Exogenous prolactin given to high-yielding dairy cows either before or after peak yield fails to alter their lactation performance (K. Plant *et al.*, 1987). In goats, increasing post-milking prolactin concentrations by 100% by infusion produces a small effect on milk yield (Jacquemet and Prigge, 1991).

The major form of mature prolactin found in the pituitary of most species is a monomer of 199 amino acids, but prolactin shows a high degree of structural polymorphism, variants differing in biological activity (Sinha, 1992). Genetic variants may result from alternative splicing. Post-translationally modified forms also occur (cleaved, glycosylated, phosphorylated, deamidated, sulphated and polymeric). Binding proteins for prolactin have been described in the milk of rabbits, sheep, goats and humans (Postel-Vinay *et al.*, 1991). The physiological significance of prolactin variants in lactation is not known, but the rat mammary gland processes prolactin (Baldocchi *et al.*, 1992)

11.2.2 GROWTH HORMONE

In contrast to prolactin, relatively little is known about growth hormone and lactation in species other than ruminants, in part because suitable long-acting inhibitors of growth hormone have been unavailable until recently. A small increase in plasma levels of growth hormone may occur during suckling in the rat (Sar and Meites, 1969; Chen *et al.*, 1974), although levels remain relatively constant and independent of suckling throughout lactation (Nagy *et al.*, 1986a). However, immunoneutralization of rat growth hormone can reduce milk secretion (Madon *et al.*, 1986b; Flint *et al.*, 1992). The importance of growth hormone increases with stage of lactation and can be seen most clearly when prolactin is also suppressed. Suckling has no effect on growth hormone release in humans (Bryant and Greenwood, 1972) or dogs (Tsushima *et al.*, 1971) and a growth hormone-deficient human can breast feed successfully (Rimoin *et al.*, 1968). In the pig there is a slight rise in growth hormone levels in early lactation (Dehoff *et al.*, 1986).

In ruminants, growth hormone and not prolactin is of principal importance in galactopoiesis. An increase in plasma levels of growth hormone occurs in response to milking in the sheep (Martal, 1975), goat (Figure 11.2; Hart and Flux, 1973) and cow (Hart, 1983). Throughout lactation growth hormone levels are raised and are correlated positively with milk yield (Hart *et al.*, 1978, 1979; Hart, 1983; Barnes *et al.*, 1985; Kazmer *et al.*, 1985), are higher in high-yielding dairy cows than beef cows (Bines and Hart, 1977; Hart *et al.*, 1978, 1979) and are higher in dairy cows selected for high milk yield than in low-yielding dairy cows (Barnes *et al.*, 1985; Kazmer *et al.*, 1986). The increase in growth hormone appears to be an adaptation to the increase in metabolic load of lactation and the relative decrease in glucose. Food restriction of cows increases growth hormone (Athanasiou and Phillips, 1978; Katzmer *et al.*, 1985) and the high growth hormone levels in dairy cows are reduced to those of lactating beef cows when they are restricted fed to equalize the milk production (Hart, 1983). Since growth hormone is lipolytic and antilipogenic, these changes in growth hormone in

high-milk-producing ruminants occur in response to metabolic stimulants related to the production of milk (Bauman and Currie, 1980) rather than directly to the suckling stimulus. Indeed, in the goat, growth hormone continued to be released in response to the milking of a denervated udder while the prolactin response, which is mediated via teat stimulation alone was abolished (Figure 11.2; Hart and Linzell, 1977), and the changes in growth hormone only appear to occur in response to milk removal (Hart and Morant, 1980).

Growth hormone secretion is regulated by the hypothalamus through the inhibitory somatostatin and the stimulatory growth hormone-releasing hormone, both in turn regulated by biogenic amines or neuropeptides originating within the central nervous system (Baile *et al.*, 1986). In cows and sheep, elevation of endogenous growth hormone secretion with growth hormone-releasing hormone results in increased milk yield (Baille *et al.*, 1986; Hart *et al.*, 1985).

The importance of growth hormone in the control of milk production in the ruminant was first shown by Folley and Young (1938), and later in growth hormone replacement studies in hypophysectomized sheep and goats (Cowie, 1966, 1969; Cowie *et al.*, 1980), and is now being exploited commercially in cattle using recombinant DNA-derived bovine growth hormone (Bauman *et al.*, 1985; Eppard *et al.*, 1987; Flint, 1987; Tucker and Merkel, 1987). However, it is still unclear whether any actions of growth hormone on lactation are direct at the mammary gland or whether all are indirect, partitioning on nutrients for milk secretion away from body stores (homeorrhesis, Bauman and Currie, 1980). It has proved impossible to show binding of growth hormone to mammary microsomes by conventional methods (Akers, 1985), but two studies in cattle have shown messenger RNA for growth hormone receptors in the mammary gland: Glimm *et al.* (1990) used *in situ* hybridization, while Hauser *et al.* (1990) used solution hybridization–nuclear protection assays. Nevertheless, close arterial infusion of growth hormone into one mammary gland in sheep and goats did not affect milk yield until the dose was high enough to increase peripheral concentrations, when milk yield was increased in both mammary glands (McDowell *et al.*, 1987). By contrast, close arterial infusion of the growth hormone-dependent insulin-like growth factor I into one mammary gland of goats increased both blood flow and milk yield in the infused gland (Prosser *et al.*, 1990), an effect not seen when insulin-like growth factor I was given systemically: it presumably arrived at the gland complexed to insulin-like growth factor-binding proteins (S.R. Davis *et al.*, 1989). Flint *et al.* (1992) were unable to replace growth hormone by systemic insulin-like growth factors I or II, despite normalizing serum concentrations, in maintaining the milk yield of lactating rats in which growth hormone was immunoneutralized. The mammary gland contains type 1 and type 2 receptors for the insulin-like growth factors. In addition, messenger RNA for insulin-like growth factor I is present in mammary glands (L.J. Murphy *et al.*, 1987), largely in the stromal compartment (Hauser *et al.*, 1990). The possibility therefore exists of local action of locally produced insulin-like growth factors. Kleinberg *et al.* (1990) found in hypophysectomized, oestrogen-treated rats that mammary growth and the mammary gland content of messenger RNA for insulin like growth factor I are stimulated by local implants of growth hormone but not prolactin.

Thus, growth hormone plays an important role in lactation in ruminants. It may act coordinately at a number of sites, directly or via the insulin-like growth factors, to partition substrates to the mammary gland but also, possibly via local insulin-like growth factor production, to stimulate blood flow and/or to produce local metabolic effects. In other species, the role of growth hormone in lactation requires further investigation. The

studies of Flint *et al.* (1992) in rats indicate that it should not be neglected.

11.2.3 INSULIN

Lactation is generally agreed to be a hypoinsulinaemic state. Insulin levels tend to be lower in lactating than in non-lactating rats (Sutter-Dub *et al.*, 1974; A.M. Robinson *et al.*, 1978; Marynissen *et al.*, 1983; Jones *et al.*, 1984; Burnol *et al.*, 1986; Hubinont *et al.*, 1986; Mizoguchi and Imamichi, 1986) and cows (Hart *et al.*, 1978; Lomax *et al.*, 1979; Blum *et al.*, 1985; Denbow *et al.*, 1986; Gerloff *et al.*, 1986; Sutton *et al.*, 1986). Insulin also tends to be higher in low- than in high-yielding cows (Hart *et al.*, 1978, 1979; Bines and Hart, 1982), and in beef cows levels of insulin increase to normal at weaning (Rutter and Manns, 1987).

Lactation induces a state of negative energy balance in many species, especially in early lactation, resulting in a decrease in blood glucose. This, together with increased insulin clearance (Jones *et al.*, 1984), may lead to the lower insulin concentrations. In addition, in ruminants the pancreatic response to glucose and propionate infusion *in vivo* is diminished (Lomax *et al.*, 1979). Madon *et al.* (1988) found no difference in the insulin response to glucose *in vitro* in islets of Langerhans from virgin and lactating rats. However, Hubinont *et al.* (1986), comparing lactating with non-lactating rats at 14 days after delivery, found reduced insulin output in response to secretagogues in pancreatic tissue from lactating rats.

In addition to hypoinsulinaemia, tissue response to insulin is altered in lactation (Vernon, 1989). In both rats and ruminants insulin action on adipocytes is impaired in the lactating animal. The rat mammary gland is responsive to insulin, while the ruminant mammary gland appears to be refractory to the acute effects of insulin (Vernon, 1989), despite apparently similar requirements for insulin across species to maintain the synthesis of milk components *in vitro* (Vonderhaar and Ziska, 1989).

11.2.4 CORTICOSTEROIDS

Plasma levels of cortisol may (Koprowski and Tucker, 1973a,b; Dunlap *et al.*, 1981a,b; Ellincott *et al.*, 1981; Whisnant *et al.*, 1985, 1986a; Spicer *et al.*, 1986a) or may not (Carruthers and Hafs, 1980; G.L. Williams *et al.*, 1984; Faltys *et al.*, 1987) increase in response to suckling or milking in the cow. Overall, plasma levels of cortisol during lactation in the cow are similar to or only slightly higher than non-lactating cows throughout the day (Dunlap *et al.*, 1981b; Keech *et al.*, 1983; Faltys *et al.*, 1987). In both the cow (Keech *et al.*, 1983; Faltys *et al.*, 1987) and rat (Gala and Westphal, 1965) there is a slight decrease in the binding capacity of corticosteroid-binding protein, while in the rat there may also be a small increase in corticosterone levels, but only during the morning, in lactating versus non-lactating rats (Voogt *et al.*, 1969; Stern *et al.*, 1973). The adrenal response to stress does appear to be reduced in the lactating rat (Stern *et al.*, 1973), although a sustained release of corticosterone can occur in lactating rats following olfactory stimulation from hungry pups (Zarrow *et al.*, 1972). Alternatively, there is little effect of lactation on corticosteroids in the pig (Ash and Heap, 1975).

In most species, adrenalectomy inhibits lactation (Cowie, 1966; Cowie *et al.*, 1980). A requirement for adrenal corticoids for the synthesis of milk components is also shown by *in vitro* culture of mammary tissue with biphasic effects on α-lactalbumin (Cowie *et al.*, 1980; Vonderhaar and Ziska, 1989). The rabbit is an apparent exception since prolactin can maintain milk yield after hypophysectomy and adrenocorticotrophin has no additional effect (Cowie *et al.*, 1969). Nevertheless, the requirements of the mammary gland for corticoids to maintain milk production appear to be met without any major alterations in plasma concentrations. In rats,

in extended lactation (Flint *et al.*, 1984), corticosteroids may become limiting, although cortisol inhibits milk ejection. The effect on lactation may result in part from suppression of the anabolic activity of adipocytes.

The rat mammary gland contains both type I (mineralocorticoid) and type II (glucocorticoid) receptors, but stimulatory actions of glucocorticoids on milk protein synthesis are exerted solely through the latter (Quirk *et al.*, 1986). Another level of control in rats is the metabolism of glucocorticoids to inactive derivatives by 11β-hydroxysteroid dehydrogenase. The activity of the enzyme is reduced in lactation (Quirk *et al.*, 1990a,b)

11.2.5 THYROID HORMONES

The thyroid hormones, triiodothyronine (T3) and thyroxine (T4), increase in lactation after an initial decrease after parturition in the goat (Riis and Madsen, 1985), the cow (Hart *et al.*, 1978; Gerloff *et al.*, 1986; Khurana and Madan, 1986) and the buffalo (Khurana and Madan, 1986). The percentage of free T3 and T4 remained constant in the cow and was negatively correlated with the levels of non-esterified fatty acids and positively correlated with cholesterol (Hart *et al.*, 1978; Gerloff *et al.*, 1986), consistent with a thyroid hormone response to the negative energy balance of the lactating dairy cow. In humans, T3 levels were normal, but both free and bound T4 levels were lower and thyroid hormone binding globulin (TBG) was higher in lactating humans (Iwatani *et al.*, 1987). Basal T4 levels are reported to be low in agalactic mares (F.N. Thompson *et al.*, 1986), suggesting that a lack of T4 may adversely affect milk production.

The biologically active T3 is produced mainly in peripheral tissues, by 5′-monodeiodination of T4. Treatment of lactating dairy cows with recombinant derived bovine growth hormone produced a twofold increase in mammary tissue thyroxine-5′-monodeiodinase activity with no change in this activity in liver or kidney or in serum concentrations of T4 or T3 (Capuco *et al.*, 1989). In the mammary gland *in vitro* T3 selectively enhances α-lactalbumin synthesis in the mouse (Vondehaar and Ziska, 1989) and stimulates casein synthesis in rabbits (Houdebine *et al.*, 1978).

11.2.6 ADRENALINE AND NORADRENALINE

Very limited evidence suggests that there may be a transient increase in plasma levels of adrenaline and noradrenaline in response to suckling or milking in the rat (C. Clapp *et al.*, 1985), sheep (Barowicz, 1979; Ewy and Barowicz, 1981), goat (Kuanjshkekova, 1976). These catecholamines together with adenosine act through membrane receptors on adipocytes via a cascade controlling lipolysis (Vernon *et al.*, 1991). The transmission of signals by the adrenergic–adenosine signal transduction cascade is altered in lactation to produce lipid mobilization as an adaptation providing substrates to the mammary gland. The components of the cascade are under complex chronic endocrine control by growth hormone, insulin, glucocorticoids and possibly other hormones (thyroid hormones, sex steroids), which may vary both between species and with tissue depot within a species (Vernon, 1991). Direct actions on the mammary gland may also occur. The β-agonist cimaterol promotes lipolysis and protein synthesis in the mammary gland of lactating rats (Choi *et al.*, 1992).

11.2.7 RELAXIN

Relaxin is a homologue of insulin and plays an important role in pregnancy maintenance being produced by the corpus luteum and/or placenta (Kemp and Niall, 1984; Weiss, 1984). Although early studies suggested that suckling in the sow and ewe resulted in an increase in plasma levels of immunoreactive relaxin (Bryant and Chamley, 1976; Afele *et al.*, 1979; Whitely *et al.*, 1985) this has not

been confirmed. In the dog (Steinetz *et al.*, 1987), and in some studies the sow (Afele *et al.*, 1979; Whitely *et al.*, 1985), relaxin is detectable in plasma throughout lactation, but other studies find levels of relaxin to decline rapidly after parturition and remain undetectable throughout lactation in rats (Sherwood, 1980), pigs (Sherwood *et al.*, 1975, 1981; Kendall *et al.*, 1983), horses (D.R. Stewart and Stabenfeldt, 1981) and cats (D.R. Stewart and Stabenfeldt, 1985). However, in the guinea pig the lactating mammary gland is a source of relaxin detectable by immunocytochemistry and Northern analysis (Peaker *et al.*, 1989). Since circulating relaxin is low in the lactating rat, the inhibitory effects of relaxin on oxytocin release from the rat hypothalamus *in vivo* (Summerlee *et al.*, 1984; O'Byrne *et al.*, 1986) would appear to represent an effect of relaxin or similar compounds released within the hypothalamus rather than an effect of peripheral levels of relaxin.

11.2.8 CONCLUSIONS

Lactation results in changes in the serum concentrations of several hormones and these changes are in general most marked in early to mid-lactation. Most notably, prolactin and growth hormone concentrations are raised while insulin or thyroid hormones fall. With the possible exception of growth hormone, these hormones act via receptors on mammary cells to promote the synthesis of milk components, but their net effect is also to partition nutrients towards the mammary gland and away from storage depots such as fat (homeorrhetic control, Bauman and Currie, 1980). It is also increasingly evident that local controls operate within the mammary gland and other tissues to interact with the endocrine changes. Local controls include alterations in blood flow, in receptors, in intracellular signalling, in the metabolism of hormones or in the production of local effector molecules, both hormones and growth factors. Good data are available for relatively few species and already suggest species differences in the ways that hormonal control of lactation is exercised.

11.3 OXYTOCIN AND THE MILK EJECTION REFLEX

During lactation milk is secreted continuously but not necessarily at a constant rate into the alveoli of the mammary glands. The milk is made available to the suckling young by the action of oxytocin causing contraction of the myoepithelial cells surrounding the alveolus to expel the milk into the collecting ducts, cisterns and galactophores from which it can be withdrawn by the sucking young. This milk ejection reflex (MER) appears to be common to all mammals even though the pattern of nursing varies enormously between species. This variation is determined in part by the rate of milk production and the time taken for the mother to secure an adequate food supply.

The interval between nursing episodes ranges from alternate days in the tree shrew (R.D. Martin, 1966) to about once per day in the rabbit (Zarrow *et al.*, 1965) and 20 or more times in the laboratory rat (Lincoln *et al.*, 1973). The total duration of nursing (defined in terms of the time spent attached to the nipples) also varies from around 3 min per day in the rabbit to 18 h per day in the rat (Lincoln, 1983). Within species, the pattern of nursing also varies considerably, as illustrated during lactation in humans, in whom nursing intervals vary from every 20 min in the hunter-gatherer !Kung tribe of Botswana and Namibia to the 'westernized' pattern of suckling with 3- to 16-h intervals.

In spite of this great variation in suckling and nursing patterns, it is clear that the ejection of milk from the mammary gland occurs as a result of the release of oxytocin from the neurohypophysis (Figure 11.3). An increase in oxytocin measured directly in plasma by either bioassay or radioimmunoassay or by

recording increases in intramammary pressure, a response specific to oxytocin, has been demonstrated in the wallaby (Lincoln and Renfree, 1981a,b), rat (e.g. Lincoln *et al.*, 1973; Higuchi *et al.*, 1985; Grosvenor *et al.*, 1986; Samson *et al.*, 1986), guinea pig (I.C.A.F. Robinson *et al.*, 1981), rabbit (Bisset *et al.*, 1970; Summerlee *et al.*, 1986), dog (Eriksson *et al.*, 1987), pig (Folley and Knaggs, 1966; Forsling *et al.*, 1979; Ellendorf *et al.*, 1982), goat (Folley and Knaggs, 1966; A.S. McNeilly, 1972) sheep (Fuchs *et al.*, 1987), cow (Folley and Knaggs, 1966; Lawson and Graf, 1968; Cleverley and Folley, 1970; Forsling *et al.*, 1974; Gorewit, 1979; Schams *et al.*, 1984) and human (Cobo *et al.*, 1967; Coch *et al.*, 1968; Cobo, 1974; Weitzman *et al.*, 1980; Lucas *et al.*, 1980; A.S. McNeilly *et al.*, 1983b; Leake and Fisher, 1985; Amico and Finley, 1986; Johnston and Amico, 1986). Oxytocin levels also increase in the cerebrospinal fluid of the sheep brain during suckling (Kendrick *et al.*, 1986). There is no evidence for a concomitant release of vasopressin during MER in any species (Bisset *et al.*, 1970; Wakerley *et al.*, 1973; Bruhn *et al.*, 1981; Johnston and Amico, 1986; Meyer *et al.*, 1987).

The pattern of oxytocin release appears at first sight to be variable between species, but much of this variation appears to be due to measurement of oxytocin in blood samples collected too infrequently during the nursing period. Since the half-life of oxytocin in the circulation is about 1.5 min, sampling frequencies of more than 1 min would give a false pattern of release.

In studies in which sampling frequency is adequate, two patterns of oxytocin release are seen (Figure 11.3). In the rabbit (Fuchs *et al.*, 1984; Summerlee *et al.*, 1986; Figure 11.2a) and cow (Folley and Knaggs, 1966; Sibaja and Schmidt, 1975; Gorewit, 1979; Sagi *et al.*, 1980; Gorewit *et al.*, 1983; Mayer *et al.*, 1984; Schams *et al.*, 1984) the release of oxytocin appears to be sustained for most of the nursing period, although the amount released in the later stages of the milking period in the cow may decline (Gorewit, 1979; Schams *et al.*, 1984).

In contrast to this sustained release of oxytocin, in the rat (Higuchi *et al.*, 1985, 1986; Mayer *et al.*, 1986), goat (A.S. McNeilly, 1972), guinea pig (I.C.A.F. Robinson *et al.*, 1981), pig (Ellendorf *et al.*, 1982), sheep (Fuchs *et al.*, 1987) and human (Lucas *et al.*, 1980; A.S. McNeilly *et al.*, 1983b) oxytocin is released in a pulsatile manner during the period of nursing (Figure 11.3).

Recent studies in which recordings of the electrical activity of the oxytocin neurosecretory cells in the paraventricular nucleus of the hypothalamus in conjunction with measurement of oxytocin release suggest that, in the rat, a single milk ejection, which occurs over a 5- to 20-s period every 2–5 min during the nursing period is associated with a single coordinated burst of firing of the oxytocin neurosecretory cells (see Summerlee and Lincoln, 1981; Lincoln, 1983; Lincoln *et al.*, 1985). This leads to a single-bolus release of oxytocin from the posterior pituitary, resulting in a pulse of oxytocin in the plasma (Higuchi *et al.*, 1985, 1986; Meyer *et al.*, 1987). Since the next episode of coordinated firing of the oxytocin neurosecretory neurones in the rat does not occur for 2–5 min, and because of the short half-life of oxytocin in the circulation, plasma concentrations of oxytocin return to baseline before the next pulse release of oxytocin. However, in the rabbit during nursing the interval between bursts of coordinated oxytocin cell neurosecretory activity resulting in oxytocin release is less than 1 min (Paisley and Summerlee, 1984), and so each release of oxytocin will occur before plasma oxytocin levels have declined from the previous release of oxytocin (Figure 11.3; Summerlee *et al.*, 1986). It is probable, therefore, that the different patterns of oxytocin release seen during suckling are related to differences in the time intervals between the coordinated firing of the oxytocin neurosecretory cells in the paraventricular nucleus.

The amount of oxytocin released appears

to increase during early lactation to a peak around mid-lactation in the rat (Sutherland *et al.*, 1986), rabbit (Fuchs *et al.*, 1984; Summerlee *et al.*, 1986) and sheep (Fuchs *et al.*, 1987) and then decline, while in humans there does not appear to be any significant change throughout lactation (Lucas *et al.*, 1980; A.S. McNeilly *et al.*, 1983b; Johnston and Amico, 1986).

In the majority of studies, there is a large variation in the amount of oxytocin released and little or no relationship has been found between the amount released and the milk volume or flow of milk in the cow (Gorewit, 1979; Sagi *et al.*, 1980; Schams *et al.*, 1984), goat (A.S. McNeilly, 1972), sheep (Fuchs *et al.*, 1987) or human (Lucas *et al.*, 1980; A.S. McNeilly *et al.*, 1983b; Johnston and Amico, 1986). This suggests that milk ejection, i.e. the abrupt contraction of the myoepithelial cells, occurs once oxytocin concentrations have increased above a threshold (Schams *et al.*, 1984; Lincoln *et al.*, 1985). As a consequence, while an increase in intramammary pressure can be achieved by increasing the dose or amount of oxytocin given as bolus injections, thus forming the basis of the oxytocin bioassays (e.g. Tindal and Yokoyama, 1962; Bisset *et al.*, 1970; Lincoln *et al.*, 1973), oxytocin given as continuous infusions tend to produce wave-like contractions of the myoepithelial cells which are not synchronized between adjacent mammary glands (Lincoln *et al.*, 1985). Indeed, only by giving small bolus injections of oxytocin is it possible to reproduce the intramammary pressure changes seen during nursing in the wallaby (Lincoln and Renfree, 1981a,b), grey seal (Lincoln *et al.*, 1983) and human (Cobo *et al.*, 1967, 1968).

The sensitivity of the mammary gland to oxytocin may change during lactation. In the rat, the amount of milk released is greater on day 13 than day 8 of lactation, but this appears to be due to a decrease in mammary resistance (Lau and Henning, 1987) since the amount of oxytocin released appears to be similar (Sutherland *et al.*, 1986). In marsupials, on the other hand, there is a clear change in sensitivity of the mammary gland to oxytocin (Lincoln and Renfree, 1981a,b). In the agile wallaby, the mammary gland can be induced to contract in the early stages of lactation to injections or infusions of oxytocin which are ineffective at later stages of lactation. During early lactation, when the young joey is continuously attached to the nipple, only a very little oxytocin is required for milk ejection. As the joey grows so the sensitivity of the mammary gland to which it is attached declines. When the joey leaves the pouch, returning to suckle, suckling will release a large amount of oxytocin to eject milk from the older glands. This large release of oxytocin would have only a limited effect on the mammary gland to which a younger joey is simultaneously attached since the pressure response reaches a plateau at very low concentrations of oxytocin. Thus, by altering the sensitivity of the mammary gland to oxytocin, the wallaby has allowed the simultaneous suckling of a large and a small joey without necessarily altering the amount of oxytocin released in response to suckling (Lincoln and Renfree, 1981a,b; Lincoln, 1983).

The release of oxytocin in the rat occurs at regular intervals of 2–10 min, but only when the rat shows slow-wave sleep (Lincoln *et al.*, 1980), the suckling stimulus being sleep inducing in the rat (Lincoln, 1983). Milk ejections occur at regular intervals during this sleep phase provided the suckling stimulus is maintained, and rat pups remain attached to the nipples for up to 18 h per day (Lincoln *et al.*, 1973). In other species sleep is clearly not a prerequisite for milk ejection (Poulain *et al.*, 1981), although rabbits and guinea pigs do enter a 'trance-like' state during their periods of nursing (Lincoln, 1983).

In the rat (Lincoln, 1983) and guinea pig (I.C.A.F. Robinson and Jones, 1982), pups suckle vigorously after milk ejection has occurred and no oxytocin appears to be re-

leased in response to exteroceptive stimuli in these species. In the pig, oxytocin release and milk ejection may be initiated by either the mother calling the litter or the piglets squealing at the mother (Ellendorf *et al.*, 1982). The piglets often massage or butt the udder for 2–4 min before oxytocin release, and oxytocin release is associated with fast grunting in the sow before milk ejection when the piglets become quiet while suckling (Ellendorff *et al.*, 1982). It is possible therefore that oxytocin release may be induced occasionally before the onset of suckling. A similar release of oxytocin may occur before teat or nipple stimulation in cows, as a result of washing of the udder (Cleverley and Folley, 1970; Gorewit, 1979; Mayer *et al.*, 1984, 1986; Schams *et al.*, 1984) or in response to washing the udder and the sound and exteroceptive stimuli of the young goats (A.S. McNeilly, 1972) or ewes (Fuchs *et al.*, 1987) and in humans in response to preparations for nursing, in particular the cry of the baby (A.S. McNeilly *et al.*, 1983b). Indeed, spontaneous milk ejections may occur at regular intervals unrelated to suckling in humans during lactation (A.S. McNeilly and McNeilly, 1978). Oxytocin release and the milk ejection reflex may be inhibited by emotional or other forms of stress (Cross, 1955; Lincoln, 1983). This has been clearly demonstrated in the rabbit (Cross, 1955), pig (Ellendorff *et al.*, 1982), cow (Bruckmaier *et al.*, 1992) and human (I. Newton, 1961). In cattle, the injection of adrenaline at the time of milking reduced milk yield by restricting blood flow to the mammary gland without affecting oxytocin release (Gorewit and Aromando, 1985). Inhibitory effects of adrenaline are mediated by α-adrenergic receptors, while milk flow rates are increased by β-adrenergic agonists (Bruckmaier *et al.*, 1991).

It is clear that in all mammalian species studied milk ejection occurs in response to a suckling-induced or -related release of oxytocin from the posterior pituitary as a result of the coordinated firing of oxytocin neurosecretory cells in the paraventricular nucleus of the hypothalamus. In most species the pattern of oxytocin release is pulsatile, but when a sustained release does occur this is probably due to the interval between firing of the oxytocin neurones being shorter than the half-life of oxytocin in blood. In some species the release of oxytocin may occur before teat or nipple stimulation and can be inhibited by stress. It appears that in most, if not all species, oxytocin is required for milk ejection. Studies which failed to demonstrate a release of oxytocin even though milk yields were normal involved blood samples collected too infrequently. Where frequent samples are collected, the failure to demonstrate a release of oxytocin during nursing is always associated with a reduced or absent milk flow (Ellendorf *et al.*, 1982; Schams *et al.*, 1984).

11.4 OVARIAN ACTIVITY DURING LACTATION

During lactation the suckled offspring stimulates milk production and milk ejection but, in most mammalian species, the suckling stimulus also delays either the resumption of ovulation or, if ovulation takes place, delays the time of implantation. In this way the interbirth interval is extended, allowing the suckling young to grow to a point where natural weaning is occurring and a reduction or absence of milk will not adversely affect the health of the growing young. In many species (e.g. cow, monkey, human) suckling prevents ovulation, in others implantation is delayed after a post-partum ovulation (e.g. rat, marsupials), while in species such as the marmoset monkey and sheep there may be a very short delay before ovulations resume. In seasonal breeders, such as the sheep, births normally occur at the end of the natural breeding season and any lactational anoestrous period coincides with seasonal anoestrus. Thus, the impact of suckling on resumption of ovarian cyclicity is almost insignificant compared with some mainly

non-seasonal breeders such as the primate, in which extended periods up to 4 years of suckling-induced infertility may occur. A final consideration is that in some species, such as the rat and many marsupials, the young are born at a very immature state, perhaps equivalent to birth of human infant at 4–6 months of the 9-month gestation, and a delay before the next pregnancy occurs or becomes sufficiently established to have a detrimental effect on the concurrent lactation is essential. In view of these different strategies, it is not possible to review the changes in ovarian activity and gonadotrophin secretion as a whole, but it is necessary to discuss the strategies of individual species before drawing conclusions as to possible common pathways involved in the suckling-induced inhibition of ovarian activity.

11.4.1 RAT

Within 24–36 h of parturition, the exact timing depending on the time of parturition (Everett, 1961; J.R. Connor and Davis, 1980a,b), a post-partum oestrus and ovulation occurs in the rat whether the rat is or is not lactating. In the natural situation, it is probable that most conceptions occur at this time (Bennett and Vickery, 1970; Conaway, 1971), although more ejaculations are necessary for conception to occur (H.N. Davis and Connor, 1980). Although the lactating female is now also pregnant, implantation is delayed for 5–7 days compared with oestrous cycles in the non-lactating rat (Conaway, 1971), during which time the blastocyst is in a metabolically quiescent state (Gosden *et al.*, 1981). Parturition then occurs around day 28, a time when natural weaning is occurring (see A.S. McNeilly, 1987a). In the absence of mating or lactation the next oestrous occurs 5–6 days after parturition, while suckling delays oestrus and ovulation for up to 20 days with natural litter sizes (Rothchild, 1960, 1981; Maneckjee and Moudgal, 1975; van der Schoot *et al.*, 1978; Sodersten *et al.*, 1983), a delay which can be increased or decreased by increasing or decreasing the litter size (Rothchild, 1960; van der Schoot *et al.*, 1978). Suckling will only delay oestrus if the suckling stimulus is vigorous during the later stages of lactation, if for instance the pups are hungry (Rothchild, 1960; van der Schoot *et al.*, 1978; Lindblom *et al.*, 1985). Whether or not conception occurs at the post-partum oestrus in lactating rats, the corpora lutea formed are maintained throughout lactation, increasing in weight and progesterone production until mid-lactation, around day 10 (Rothchild, 1960; Grota and Eik-Nes, 1967; Tomogane *et al.*, 1969; Yoshinaga *et al.*, 1971; M.S. Smith and Neill, 1977; van der Schoot *et al.*, 1978; Gosden *et al.*, 1981; Taya and Greenwald, 1982; Taya and Sasamoto, 1987). The corpora lutea of pregnancy are also maintained, although with a much reduced activity (Taya and Greenwald, 1982). Both sets of corpora lutea are dependent on prolactin for the maintenance of progesterone secretion (Yoshinaga, 1974; Ford and Yoshinaga, 1975; M.S. Smith *et al.*, 1976; van der Schoot *et al.*, 1978; Taya and Greenwald, 1982) and, since the amount of prolactin available is related to the suckling intensity and hence litter size, the amount of progesterone secreted is indirectly related to litter size (Yoshinaga *et al.*, 1971; Coppings and McCann, 1979; Taya and Greenwald, 1982). Although corpora lutea are maintained throughout lactation, follicular development is suppressed. No large antral follicles are present and oestradiol levels are low during the first half of lactation (M.S. Smith and Neill, 1977; van der Schoot *et al.*, 1978; Gosden *et al.*, 1981; Taya and Greenwald, 1982; Uilenbroek *et al.*, 1982; Sodersten *et al.*, 1983; Taya and Sasamoto, 1987; Lux-Lantos *et al.*, 1990). No compensatory ovarian hypertrophy occurs after unilateral ovariectomy at this time (Mena *et al.*, 1974; van der Schoot and Greef, 1986a) and no ovulations will occur after treatment with an ovulatory dose of either LH or hCG (Taya and Greenwald,

1982; Taya and Sasamoto, 1987). Follicle growth resumes around day 10 of lactation, and plasma levels of inhibin (Taya *et al.*, 1989; Taya and Sasamoto, 1991; Yohkaichiya *et al.*, 1991) and oestradiol increase slightly at this time, allowing oestrogen-dependent implantation of the blastocysts to occur (Psychoyos, 1973; M.S. Smith and Neill, 1977; Taya and Greenwald, 1982; Taya and Sasamoto, 1987, 1991). Major follicle growth and the development of healthy antral follicles does not occur in rats with normal litter sizes until around days 17–18 (Taya and Greenwald, 1982; van der Schoot and Greef, 1986a,b; Taya and Sasamoto, 1987,1991). Although plasma oestradiol levels may not increase significantly, ovarian compensatory hypertrophy after unilateral ovariectomy will now occur in these later stages of lactation (Mena *et al.*, 1974; van der Schoot and Greef, 1986a,b).

11.4.2 PIG

Oestrus cyclicity in the pig is delayed for up to 6 weeks during lactation, and the delay is not necessarily dependent on the number of piglets suckling since lactational anoestrus can be maintained with as few as one or two piglets (Parvizi *et al.*, 1976; Kuravongkrit, 1984). However, the number of piglets and hence the suckling intensity can markedly affect the time from weaning to first oestrus and ovulation (Fahmy *et al.*, 1979), and this interval is also reduced with longer lactations, presumably because of a reduction in the suckling intensity of the older piglets (Self and Grummer, 1958; Moody and Speer, 1971; Cole *et al.*, 1975). A reduction in the suckling duration per day is achieved by removing the litter for up to 12 h (partial weaning) from the second to third week of lactation (D.M. Smith, 1961; Walker and England, 1978; L.H. Thompson *et al.*, 1981; Kirkwood *et al.*, 1983; Henderson and Hughes, 1984; Stevenson and Davis, 1984; E.A. Newton *et al.*, 1987) can result in the occurrence of oestrus during lactation, while split weaning, in which a portion of the litter is completely weaned, will increase the number of sows exhibiting early oestrus after weaning (Stevenson and Britt, 1981; Cox *et al.*, 1983; Henderson and Hughes, 1984; Stevenson and Davis, 1984).

Since first oestrus normally occurs between 12 and 20 days after parturition in the non-lactating sow, a proportion of the delay in resumption of oestrous cycles is due to recovery of the hypothalamo-pituitary axis from the suppressive effects of pregnancy (Elliot *et al.*, 1980). However, even in the absence of lactation, abnormal luteal function can occur for up to 6 weeks after parturition (Kuravongkrit *et al.*, 1983a,b). During lactational anoestrus no corpora lutea and only a small population of large follicles are present in the ovary (Lauderdale *et al.*, 1965; Palmer *et al.*, 1965a,b; Crighton and Lamming, 1969; Asch and Heap, 1975; Stevenson *et al.*, 1981; Cox and Britt, 1982a; Duggan *et al.*, 1982; Kuravongkrit *et al.*, 1982; Dyck, 1983; Edwards and Foxcroft, 1983; Foxcroft and Hunter, 1985; Shaw and Foxcroft, 1985; M.J. Martin *et al.*, 1986; De-Rensis *et al.*, 1991). There is a large population of small antral follicles in anoestrus and, with advancing lactation, the size of these follicles increases and the number undergoing atresia decreases (Palmer *et al.*, 1965b; Cox and Britt, 1982a; Kuravongkrit *et al.*, 1982; Foxcroft and Hunter, 1985) although no follicles of a size equivalent to a preovulatory follicle are usually seen (Palmer *et al.*, 1965b; Kuravongkrit *et al.*, 1982; Foxcroft and Hunter, 1985; Foxcroft *et al.*, 1987). After weaning there is a rapid increase in the number of medium and large follicles and a reduction in the number of small antral follicles with a concomitant increase in plasma levels of oestradiol (Palmer *et al.*, 1965a; Cox and Britt, 1982a; Dyck, 1983; Foxcroft *et al.*, 1987).

11.4.3 SHEEP

In natural conditions, lambing and sub-

sequent lactation occur in the spring at a time when day length is increasing, which coincides with the onset of seasonal anoestrus in most breeds of sheep. Thus, the effect of lactation on oestrous cyclicity is not clear. In suckled ewes which are either autumn lambing in which lambing occurs before the start of seasonal anoestrus, oestrous cycles resume within 3–5 weeks (Shevah *et al.*, 1975; Kann *et al.*, 1979). Lactation may (Shevah *et al.*, 1975; Kann *et al.*, 1979; Moss *et al.*, 1980; P.J. Wright *et al.*, 1980; Gregg *et al.*, 1986; Schirar *et al.*, 1989) or may not (Sharpe *et al.*, 1986; Malven and Hudgens, 1987) delay the resumption of oestrous cycles, but this variation may relate to differences between breeds of sheep and the latitude at which the studies were carried out. However, there is evidence of a reduction in fertility of lactating compared with non-lactating ewes following oestrus induction (Restall *et al.*, 1978; Rhind *et al.*, 1980).

11.4.4 COW

Both milking and suckling delay the onset of oestrous cyclicity after parturition in the cow (R.E. Short *et al.*, 1972; G.L. Williams, 1990), although suckling will cause a longer delay of up to 150 days (mean around 57 days) before first ovulation compared with 30–70 days (mean around 25 days) in milked cows (Wiltbank and Cook, 1958; Casida *et al.*, 1968; Oxenreider, 1968; R.E. Short *et al.*, 1972; Hurnick *et al.*, 1975; King *et al.*, 1976; Webb *et al.*, 1977; Bulman and Lamming, 1978; Schams *et al.*, 1978; Stevenson and Britt, 1979; Carruthers and Hafs, 1980; A.R. Peters and Lamming, 1984; Bryner *et al.*, 1990; M.G. Murphy *et al.*, 1990; Randel, 1990; Savio *et al.*, 1990a,b; Schemm *et al.*, 1990; Spicer *et al.*, 1990; G.L. Williams, 1990). In suckled cows the interval to first oestrus can be reduced if calves are removed temporarily or suckling is restricted (England *et al.*, 1973; Tribble *et al.*, 1973; J.S. Connor *et al.*, 1974; Randel *et al.*, 1976; Wetterman *et al.*, 1978; Carter *et al.*, 1980; Randel and Welker, 1981; Suzuki and Sato 1984; Fogwell *et al.*, 1986; I.A. Wright *et al.*, 1987). An increase in either milking frequency or the number of calves suckling may (H. Clapp, 1937; Wetterman *et al.*, 1978) or may not (Wetterman *et al.*, 1978; Lamming *et al.*, 1981; A.R. Peters *et al.*, 1981; Montgomery, 1982; A.R. Peters and Riley, 1982a) extend the interval to first oestrus.

The considerable variation in the delay to resumption of oestrus in both suckled and milked cows is related not only to suckling or milking patterns but is considerably influenced by environmental factors, of which photoperiod and nutrition are of major importance. Although not displaying seasonal anoestrus, non-lactating cows do show a seasonal change in ovarian follicular development (McNatty *et al.*, 1984). In parallel with this, the interval to oestrus is longer in suckled or milked cows calving in late autumn and winter than those calving in the spring or summer (Bulman and Lamming, 1978; Montgomery *et al.*, 1980; A.R. Peters and Riley, 1982b; P.J. Hansen and Hauser, 1983; P.J. Hansen, 1985). This effect can be replicated by artificial photoperiods (P.J. Hansen and Hauser, 1983, 1984) and is closely related to nutrition. Heavier and better nourished animals or those supplied with 24 supplementary dieting energy resume oestrous cyclicity earlier (Wiltbank *et al.*, 1964; Butler and Smith, 1989; Dunn *et al.*, 1969; A.R. Peters and Riley, 1982a; I.A. Wright *et al.*, 1987; Prado *et al.*, 1990; Randel, 1990), while oestrous cyclicity occurs later in poorly nourished cows (Lamming, 1966; Oxenreider and Wagner, 1971; Stevenson and Britt, 1979; I.A. Wright *et al.*, 1987) related to a reduction in body condition (I.A. Wright *et al.*, 1987; Randel, 1990).

Before first ovulation the final stages of follicular development are suppressed and plasma and milk levels of progesterone remain basal confirming the absence of ovulation (Labhetswar *et al.*, 1964; R.E. Short *et al.*, 1972; Webb *et al.*, 1977; Schams *et al.*, 1978; Lishman *et al.*, 1979; Rawlings *et al.*,

1980; Walters *et al.*, 1982; Bellin *et al.*, 1984; A.R. Peters and Lamming, 1984; Dufour and Roy, 1985; Moss *et al.*, 1985; Eduvie and Dawida, 1986; Garcia-Winder *et al.*, 1986, 1987; Jagger *et al.*, 1987; Bryner *et al.*, 1990; Perry *et al.*, 1991a,b). Plasma levels of oestradiol remain low and rise only with the development of large healthy follicles preceding the first preovulatory LH surge (Lishman *et al.*, 1979; Rawlings *et al.*, 1980; Walters *et al.*, 1982; Moss *et al.*, 1985; Jagger *et al.*, 1987; Garcia-Winder *et al.*, 1987; Spicer *et al.*, 1987a; Savio *et al.*, 1990a,b; Schemn *et al.*, 1990; Spicer *et al.*, 1990). Large healthy follicles have been observed in weaned cows by 7 days, in milked cows by 10 days and in suckled cows by 20 days post-partum (Oxenreider and Wagner, 1971; Hoffman *et al.*, 1973; Lishman *et al.*, 1979; Savio *et al.*, 1990a,b) with the percentage of non-atretic follicles in the ovary before first ovulation decreasing with time post-partum (Lishman *et al.*, 1979; Dufour and Roy, 1985). The corpus luteum of the first ovulation in both suckled and milked cows is usually short-lived (M.G. Murphy *et al.*, 1990; Savio *et al.*, 1990a; Perry *et al.*, 1991a).

11.4.5 NON-HUMAN PRIMATES

The majority of primates exhibit extended periods of suckling-induced ovarian inactivity, sometimes extending to 4 years in Old World great apes, monkeys and humans. In most species the resumption of ovarian activity has been assessed by monitoring interbirth intervals and the endocrine changes associated with the delay in resumption of ovulation are restricted to a very few species. Nevertheless, it is clear that lactation or suckling delays the resumption of ovulation in the great apes, such as the gorilla (Harcourt *et al.*, 1980) and chimpanzee (Douglas and Butler, 1970; Clegg and Weaver, 1972; Tutin, 1980), lesser Old World anthropoids such as the baboon (Rowell, 1974), various macaques (Kaufman, 1965; Fujiwara *et al.*, 1967; Vandenberg and Vessey, 1968; Drickamer, 1974; Weiss *et al.*, 1976a; Varley and Vessey, 1977; Simpson *et al.*, 1981; Wilson *et al.*, 1988; Gomendio, 1989; Nozaki *et al.*, 1990; Maeda *et al.*, 1991; Gordon *et al.*, 1992) and members of the genus *Cercopithecus* (Mallinson, 1971; Rowell, 1974) and the New World monkeys including the spider (Wolf *et al.*, 1975), howler (Carpenter, 1934) and squirrel monkeys (Travis and Holmes, 1974; Coe and Rosenblum, 1978), but not in the common marmoset (A.S. McNeilly *et al.*, 1981; S.F. Lunn and McNeilly, 1982) or cotton-top tamarins (Ziegler *et al.*, 1990). In the rhesus monkey, lactational infertility is associated with an absence of ovarian follicular development observed as the persistence of low plasma levels of oestradiol for up to 150 days post-partum (Weiss *et al.*, 1973; R.F. Williams and Hodgen, 1983; Gomendio, 1989; Gordon *et al.*, 1992), in contrast to the non-suckled monkey, in which normal follicular development and ovulation occurs by about 42 days post-partum with menses occurring 14 days later (R.F. Williams and Hodgen, 1983). In these non-suckled monkeys, the corpus luteum of pregnancy regresses soon after parturition with a resultant rapid decline in plasma levels of progesterone (Weiss *et al.*, 1973; Goodman and Hodgen, 1978; T.M. Plant *et al.*, 1980; Richardson *et al.*, 1985). In contrast, in suckled lactating mothers, the corpus luteum of pregnancy is maintained for the first 2–3 months post-partum as a result of the increased plasma levels of prolactin associated with lactation (T.M. Plant *et al.*, 1980; Schallenberger *et al.*, 1981; Richardson *et al.*, 1985) and, although plasma levels of progesterone do fall over the first few days post-partum, they are maintained above normal follicular phase levels for 2–3 months (Weiss *et al.*, 1973; T.M. Plant *et al.*, 1980; R.F. Williams and Hodgen, 1983). Luteolysis of this corpus luteum of pregnancy/lactation occurs if prolactin levels are suppressed either by weaning the infant (T.M. Plant *et*

al., 1980) or by treating the mother with bromocriptine (Schallenbelger *et al.*, 1981; Richardson *et al.*, 1985). In contrast to all primates studied so far, lactation in the common marmoset is not associated with any significant delay in the resumption of ovarian activity post-partum, even though the mothers normally have twins (A.S. McNeilly *et al.*, 1981; S.F. Lunn and McNeilly, 1982).

11.4.6 HUMANS

Breastfeeding and lactation in women can induce a period of ovarian inactivity and amenorrhoea for up to 4 years but, because of the immense variation in breastfeeding patterns, the duration of this interval is very variable and may be as little as 2–3 months even in a fully breastfeeding mother (Udesky, 1950; Perez *et al.*, 1972; R.V. Short, 1976; Delvoye *et al.*, 1978; A.S. McNeilly, 1979, 1987a; Kennedy *et al.*, 1989; Konner and Worthman, 1980; P.G. Lunn *et al.*, 1980; Andersen and Schioler, 1982; Howie and McNeilly, 1982; Brown *et al.*, 1985; Duchen and McNeilly, 1980; Gross and Eastman, 1985; Hennart *et al.*, 1981, 1985; P.G. Lunn, 1985; Rivero *et al.*, 1985, 1988; Wood *et al.*, 1985a,b; Stern *et al.*, 1986). During the period of lactational amenorrhoea, ovarian follicular development, whether monitored by changes in plasma levels of oestradiol or urinary excretion of oestrogen (Reyes *et al.*, 1972; Bonnar *et al.*, 1975; Rolland *et al.*, 1975; Andreassen and Tyson, 1976; Baird *et al.*, 1979; Duchen and McNeilly, 1980; A.S. McNeilly *et al.*, 1980, 1982a; Howie *et al.*, 1981, 1982; Howie and McNeilly, 1982; Glasier *et al.*, 1983; Gross and Eastman, 1983, 1985; Fraser *et al.*, 1989; Israngkura *et al.*, 1989; Gray *et al.*, 1990; Fink *et al.*, 1992), appears to be suppressed. Luteal function rarely resumes, although small short-term increases in oestrogen secretion lasting a few days (P.R. Lewis *et al.*, 1985; Rivero *et al.*, 1985, 1988; Shaaban *et al.*, 1990; R.V. Short *et al.*, 1991; Tay *et al.*, 1992) may occur but are usually not maintained (Howie and McNeilly, 1982; Gross and Eastman, 1985; A.S. McNeilly *et al.*, 1985; Flynn *et al.*, 1991). These increases in oestradiol are associated with the growth of follicles as visualized by ultrasound, and medium-sized non-steroidogenically active follicles are also observed (Flynn *et al.*, 1991). In non-breastfeeding women, an increase in plasma levels of oestradiol resulting from the development of a preovulatory follicle occurs within 15–30 days post partum and ovulation occurs within 30–50 days post-partum (Bonnar *et al.*, 1975; Baird *et al.*, 1979; A.S. McNeilly *et al.*, 1980; Howie and McNeilly, 1982; Howie *et al.*, 1982; Poindexter *et al.*, 1983; Gross and Eastman, 1985; Gray *et al.*, 1987).

Ovulation may occur in a proportion of humans during breastfeeding, and in some this may occur before menstruation and may result in a pregnancy (Howie and McNeilly, 1982; A.S. McNeilly *et al.*, 1983c; Gross and Eastman, 1985). In the majority, ovulation is followed by menstruation, and in 40–60% of menstrual cycles occurring while humans continue to breastfeed, the luteal phases of these menstrual cycles are associated with inadequate corpus luteum function (Delvoye *et al.*, 1980; Duchen and McNeilly, 1980; A.S. McNeilly *et al.*, 1980, 1982a,b, 1983c; Howie and McNeilly, 1982; Gross and Eastman, 1985; P.R. Lewis *et al.*, 1985; Gray *et al.*, 1990; Shaaban *et al.*, 1990), a situation similar to the resumption of menstrual cycles in a non-breastfeeding human post-partum (A.S. McNeilly *et al.*, 1980, 1982a, 1983c; Poindexter *et al.*, 1983; Gray *et al.*, 1987). Normal ovulatory menstrual cycles are more likely to occur later in lactation (A.S. McNeilly *et al.*, 1982a).

The duration of lactational amenorrhoea or infertility is directly related to the suckling activity of the infant (A.S. McNeilly, 1979, 1987a; A.S. McNeilly *et al.*, 1980, 1985; Howie and McNeilly, 1982; Thapa *et al.*, 1988; Kennedy and Visness, 1992), and this

accounts for the large variation in duration both within and between different societies. A reduction in breastfeeding, usually in terms of frequency but also in duration of each breast feed, can result in the resumption of ovarian activity even though breastfeeding continues (Rolland *et al.*, 1975; Delvoye *et al.*, 1978; Andersen and Schioler, 1982; Howie and McNeilly, 1982; Howie *et al.*, 1982; Brown *et al.*, 1985; Hennart *et al.*, 1985; Elias *et al.*, 1986; Gray *et al.*, 1990; Shaaban *et al.*, 1990; R.V. Short *et al.*, 1991). These changes in suckling pattern may occur as a result of factors influencing the baby, e.g. introduction of supplementary food (Howie *et al.*, 1982; Elias *et al.*, 1986) or the mother, in whom poor nutrition may extend the period of infertility (P.G. Lunn *et al.*, 1980; Hennart *et al.*, 1985). Suckling the baby at night may also prolong the duration of infertility (Howie and McNeilly, 1982; Wood *et al.*, 1985a,b; Elias *et al.*, 1986; Stern *et al.*, 1986) although in these circumstances suckling frequency and duration during the day also tend to be greater suggesting that it may not be an effect specific to night-time feeding (Stern *et al.*, 1986; A.S. McNeilly, 1987a).

11.5 GONADOTROPHINS

In most mammalian species for which adequate data are available, the changes in gonadotrophin secretion post-partum and during lactation are similar. Most of the variation between species appears to occur as a result of differences in the pattern of suckling, nursing or milking. The pituitary content of both LH and FSH is decreased in most species by the negative-feedback effects of the high levels of steroid hormones associated with pregnancy. After parturition, therefore, there is a finite time period during which pituitary synthesis of gonadotrophins returns to normal whether lactation occurs or not. The following sections will detail these changes in pituitary hormone content, plasma hormone concentrations and responses to ovariectomy and oestradiol.

11.5.1 FOLLICLE-STIMULATING HORMONE

The pituitary content of FSH increases to normal within a few days of parturition in the rat (M.S. Smith, 1982a, 1985; Taya and Greenwald, 1982), pig (Crighton and Lamming, 1969), sheep (Chamley *et al.*, 1976; Moss *et al.*, 1980; Crowder *et al.*, 1982; Clarke *et al.*, 1984) and cow (Labhsetwar *et al.*, 1964; Saiduddin *et al.*, 1968; Carruthers *et al.*, 1980; Cermak *et al.*, 1983; Moss *et al.*, 1985; Leung *et al.*, 1986). In the rhesus monkey, the pituitary content of FSH is decreased during early lactation but returns to normal by mid-lactation (Weiss *et al.*, 1976b). *In vitro*, pituitaries from lactating rats (M.S. Smith, 1982a, 1985) and cows (Leung *et al.*, 1986) release normal amounts of FSH in response to gonadotrophin-releasing hormone (GnRH). Plasma levels of FSH in most species return to those seen in normal non-lactating animals early in lactation (rat, M.S. Smith and Neill, 1977; M.S. Smith, 1978a,b; van der Schoot *et al.*, 1978; Taya and Sasamoto, 1980, 1987, 1990, 1991; Taya and Greenwald, 1982; pig, Stevenson *et al.*, 1981; Cox and Britt, 1982b; S. Edwards and Foxcroft, 1983; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987; E.A. Newton *et al.*, 1987; sheep, Findlay and Cumming, 1976; Fitzgerald and Cunningham, 1981; Clarke *et al.*, 1984; Schirar *et al.*, 1990; cow, Dobson, 1978; Schams *et al.*, 1978; Webb *et al.*, 1980; Lamming *et al.*, 1981; Schallenberger and Petersen, 1982; G.L. Williams *et al.*, 1982, 1983; Leung *et al.*, 1986; Spicer *et al.*, 1986b, I.A. Wright *et al.*, 1987; Bryner *et al.*, 1990; human, Reyes *et al.*, 1972; Bonnar *et al.*, 1975; Rolland *et al.*, 1975; Keye and Jaffe, 1976; Baird *et al.*, 1979; A.S. McNeilly *et al.*, 1980; Howie and McNeilly, 1982; Glasier *et al.*, 1983; Tay *et al.*, 1992) or by mid-lactation in the rhesus monkey (T.M. Plant *et al.*, 1980; Gordon *et al.*, 1992). Injections of GnRH cause the release of nor-

mal amounts of FSH in the rat (M.S. Smith, 1982a, 1985), in the pig (Stevenson *et al.*, 1981; Cox and Britt, 1982a) and in humans (Jeppson *et al.*, 1974; Le Maire *et al.*, 1974; Andreassen and Tyson, 1976; Keye and Jaffe, 1976; Delvoye *et al.*, 1978; Tay *et al.*, 1993). In the cow, the FSH response to GnRH given either as a single bolus (Stevenson and Britt, 1980) or as small repeated injections (A.R. Peters *et al.*, 1985), while unaffected by suckling early in lactation (G.L. Williams *et al.*, 1982), declined with time post-partum in relation to the increase in ovarian follicular development and plasma levels of oestradiol (Jagger *et al.*, 1987).

Ovariectomy results in an increase in FSH, but the response depends on the stage of lactation and the suckling intensity. Thus, the FSH response to ovariectomy is reduced in early lactation in the rat (Taya and Sasamoto, 1991), pig (Stevenson *et al.*, 1981) and monkey (Weiss *et al.*, 1976a,b) but returns to normal by mid-lactation, while in the cow the post-ovariectomy FSH response is normal soon after parturition (Acosta *et al.*, 1983; Hinshelwood *et al.*, 1985). The FSH response to weaning depends on the degree of ovarian activity and the amount of steroid negative feedback. If follicular development is advanced and oestradiol levels are increased then there is no change in or even a decrease in FSH (pig, Foxcroft *et al.*, 1987; E.A. Newton *et al.*, 1987; cow, I.A. Wright *et al.*, 1987), while an increase in FSH may result if weaning occurs earlier in lactation when follicular development and hence steroid and inhibin feedback are suppressed (pig, Shaw and Foxcroft, 1985; E.A. Newton *et al.*, 1987). The positive-feedback response to an oestradiol challenge is reduced or absent during early and mid-lactation in both the monkey (Yamaji *et al.*, 1971; R.F. Williams *et al.*, 1979; T.M. Plant *et al.*, 1980; Schallenberger *et al.*, 1981; Ash *et al.*, 1984; Richardson *et al.*, 1985) and humans (Baird *et al.*, 1979; Glass *et al.*, 1981; Vermer and Rolland, 1982), and although absent at day 5 is normal by day 15–17 post-partum in the cow (A.R. Peters, 1984). In humans, oestradiol has a greater negative feedback effect on FSH (Baird *et al.*, 1979).

In summary, it appears that in most species both the pituitary content and plasma concentrations of FSH return to normal soon after parturition, suggesting that a lack of FSH is not involved in the suppression of ovarian activity post-partum. However, it must be remembered that in most species there is a lack of ovarian activity with consequent lack of the ovarian negative-feedback hormones oestradiol and inhibin. Therefore, it might be expected that plasma concentrations of FSH would be higher than normal.

11.5.2 LUTEINIZING HORMONE

Luteinizing hormone is normally released in pulses, each resulting from the release of a pulse of GnRH from the hypothalamus. It is thus appropriate to discuss changes in GnRH receptors in the pituitary in relation to changes in the pituitary content of LH and plasma levels of LH. As with FSH, the time course of changes in LH during lactation are similar between species when differences in suckling or milking intensity are taken into account. For clarity some of these changes will be described for each species and common features of all species then discussed.

(a) Rat

The pituitary content of LH, LHb and gonadotrophin α-subunit mRNA abundance is decreased during early lactation, increasing to that of a dioestrous female by mid-lactation (Ford and Melampy, 1973; M.S. Smith and Neill, 1977; M.S. Smith, 1978a,b; Steger and Peluso, 1978; Taya and Greenwald, 1982; Taya and Sasamoto, 1987; L.R. Lee *et al.*, 1989a,b; M.S. Smith and Lee, 1989). This reduced pituitary content of LH is accompanied by reduced hypothalamic GnRH content (Minaguchi and Meites, 1967; M.S.

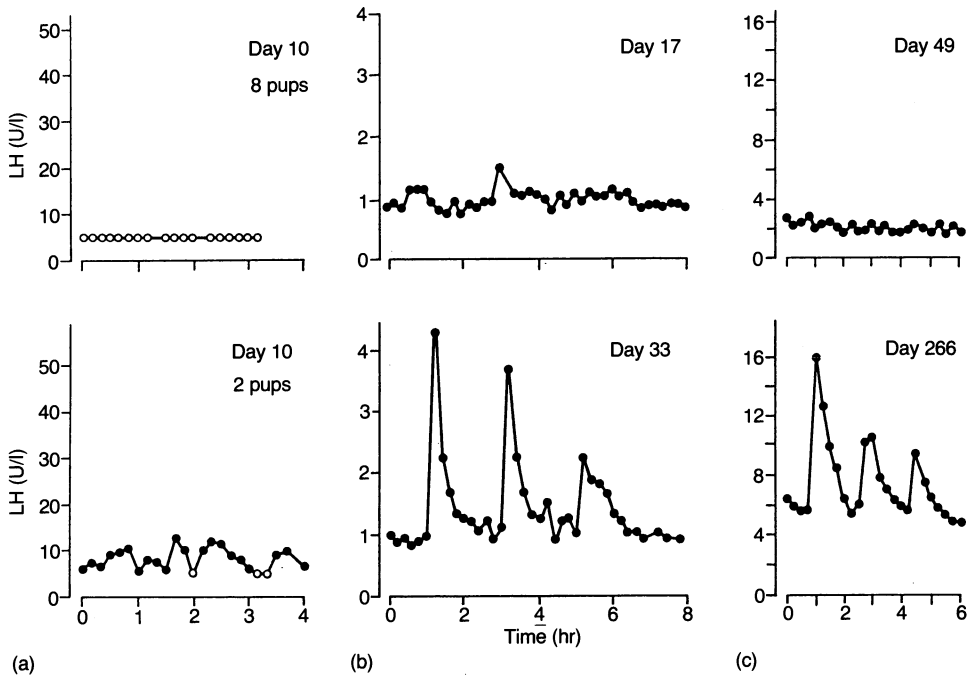

Figure 11.4 Changes in the pulsatile secretion of LH during lactation in the rat (A), cow (B) and human (C). Note that early in lactation in the cow and human, and in early to mid-lactation (day 10) in the rat suckled by a normal-size litter, the pulsatile secretion of LH is inhibited or absent. As lactation proceeds in the cow and human the pulsatile release of LH resumes and is associated with a return of ovarian follicular development. In the rat, a reduction in the suckling stimulus by reducing the number of pups from eight to two also results in an increase in the pulsatile secretion of LH. (Taken from Fox and Smith, 1984; Glasier *et al.*, 1984b; Riley *et al.*, 1981.)

Smith, 1978a) and there is a reduced or absent release of GnRH in response to the excitatory amino acid *N*-methyl aspartic acid (Pohl *et al.*, 1989). There is up to a 50% reduction in pituitary GnRH receptors (Clayton *et al.*, 1980; M.S. Smith, 1984), suggesting an absence of GnRH release from the hypothalamus, since GnRH in the rat is known to positively regulate its own receptors and GnRH receptor number reflects exposure of the pituitary to endogenous GnRH (Clayton *et al.*, 1982; Pieper *et al.*, 1982).

Basal plasma levels of LH are suppressed to around 25% of those in dioestrous (Figure 11.4; Ford and Melampy, 1973; Hammons *et al.*, 1973; Lu *et al.*, 1976a,b; M.S. Smith and Neill, 1977; M.S. Smith, 1978a,b, 1982b, 1985; van der Schoot *et al.*, 1978; Coppings and McCann, 1979; Taya and Greenwald, 1982; Uilenbroek *et al.*, 1982; Hansen *et al.*, 1983; Fox and Smith, 1984; Taya and Sasamoto, 1987, 1990, 1991; Lux-Lantos *et al.*, 1990) and increase in mid- to late lactation, around day 20, when plasma levels of progesterone decline and those of 20α-dihydroprogesterone increase (M.S. Smith and Neill, 1977; Taya and Greenwald, 1982).

These low basal levels of LH result from an absence of the normal pulsatile secretion of LH, at least on day 10 of lactation (Figure 11.4; Fox and Smith, 1984; L.R. Lee *et al.*, 1989a,b). Exogenous GnRH given as large bolus or small pulsatile injections releases LH from the pituitary both *in vitro* (M.S. Smith,

1982a, 1985) and *in vivo* (Lu *et al.*, 1976a,b; Hodson *et al.*, 1978; M.S. Smith, 1978a; Steger and Peluso, 1978; Fox and Smith, 1984; L.R. Lee *et al.*, 1989a,c) during lactation, but the amount of LH released is significantly reduced in comparison with dioestrus, and no potentiation of the LH response was seen in response to repeated injection of GnRH (Lu *et al.*, 1976a,b; Hodson *et al.*, 1978), as occurs normally during the oestrous cycle (Aiyer *et al.*, 1974). Longer treatment with pulsatile GnRH induces a rapid increase in GnRH receptors to normal (L.R. Lee *et al.*, 1989a,c), a response similar to the increase in LH pulse output, reflecting GnRH, and GnRH binding after weaning (L.R. Lee *et al.*, 1989a,b; M.S. Smith and Lee, 1989; Ciofi *et al.*, 1991). The increase in LH secretion was accompanied by an increase in LHβ and gonadotrophin α-subunit mRNA abundance (L.R. Lee *et al.*, 1989a,b) and could be reversed if suckling resumed (Maeda *et al.*, 1989).

In lactating female rats, the LH response to an oestradiol injection, which will induce the release of a preovulatory-type LH surge in the normal oestrous cycle (Legan *et al.*, 1975; Blake, 1977), is significantly reduced (M.S. Smith, 1978a; Coppings and McCann, 1979), the amount of LH released decreasing as litter size increases (Coppings and McCann, 1979). Similarly, the daily LH surges which can be induced in non-lactating rats when exposed to continuous oestradiol (Legan *et al.*, 1975) are abolished (Coppings and McCann, 1979) or dramatically reduced (Tsukamura *et al.*, 1988) during lactation. Plasma concentrations of progesterone are elevated during lactation due to the persistence of the corpus luteum of pregnancy and to the new corpora lutea occurring as a result of the post-partum ovulation. This progesterone, together with any follicular steroid production, may play a role in suppressing GnRH output, and this can be assessed by monitoring the changes in gonadotrophins after ovariectomy during lactation. When ovariectomy is performed in early lactation suckling can prevent the normal rise in serum and pituitary levels of LH and, to a lesser extent, FSH (M.S. Smith, 1978b, 1981), and the normal decrease in hypothalamic content of GnRH (Culler *et al.*, 1982a,b) and the post-ovariectomy suppression of LH and FSH can be enhanced if litter size is increased (Coppings and McCann, 1979). In contrast, when ovariectomy is performed in mid-lactation (days 10–15), suckling only reduced the increase in LH and FSH by 50% (M.S. Smith, 1981). The importance of the suckling stimulus in suppressing LH release was demonstrated in studies in which pups were separated from their mothers for 8–23 h (Isherwood and Cross, 1980; Sirinathsinghji and Martini, 1984). During the suckling period when the pups were reintroduced there was a dramatic increase in plasma levels of prolactin and rapid decrease in LH (Isherwood and Cross, 1980; Sirinathsinghji and Martini, 1984) but not FSH (Sirinathsinghji and Martini, 1984), the increase in LH associated with an increase in pulsatile LH/GnRH release (Maeda *et al.*, 1989). Although these results suggest that suckling will induce an acute decrease in LH secretion, suckling and nursing activity, in the rat is a more continuous activity with nursing periods lasting up to 18 h. The suckling activity of the pups declines throughout lactation with considerable variation during each 24 h period (van der Schoot *et al.*, 1978; Sodersten and Eneroth, 1984) and, although LH levels may be lower during a 24-h period in mothers suckling 10 rather than five pups (van der Schoot *et al.*, 1978), there is no precise relationship between suckling activity and LH release (van der Schoot *et al.*, 1978; Sodersten and Eneroth, 1984). This suggests that during lactation in the rat the effect of suckling on LH secretion is related more to a chronic alteration in secretion than a series of acute changes occurring every suckling episode. Nevertheless, in the rat, reduced basal levels of LH during lactation result from a suckling-

induced decrease in the hypothalamic content of and subsequent release of GnRH from the hypothalamus, which results in a reduced number of GnRH receptors in the pituitary and a decrease in both the synthesis and release of LH both basally and in response to both GnRH and oestradiol. A similar situation occurs in other species.

(b) Pig

During the first 3–4 weeks of lactation the pituitary content of LH (Crighton and Lamming, 1969; Cox and Britt, 1982b) and GnRH (Cox and Britt, 1982b) and basal plasma levels of LH remain low (Aherne *et al.*, 1976; Parvizi *et al.*, 1976; Dyck *et al.*, 1979; van der Wiel *et al.*, 1979; Cox and Britt, 1982b, Duggan *et al.*, 1982; Stevenson and Britt, 1979; Stevenson *et al.*, 1981; Edwards and Foxcroft, 1983; Foxcroft *et al.*, 1987; E.A. Newton *et al.*, 1987), and neither pituitary nor plasma levels of LH increase in response to ovariectomy at this stage (Crighton and Lamming, 1969; Stevenson *et al.*, 1981). The suppression of LH is associated with an absence or decrease in the pulsatile release of LH (Parvizi *et al.*, 1976; van der Wiel *et al.*, 1979; S. Edwards and Foxcroft, 1983; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987; E.A. Newton *et al.*, 1987) and is directly related to litter size (Kuravongkrit, 1984).

From the third week of lactation onwards the pulsatile secretion of LH resumes, but initially at a lower frequency than normal (Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987; Barb *et al.*, 1991; Rojkittikhun *et al.*, 1991). Plasma levels of LH in intact (Elsaesser and Parvizi, 1980; Stevenson and Britt, 1980; Stevenson *et al.*, 1981; Kirkwood *et al.*, 1984a; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987) and ovariectomized sows (Stevenson *et al.*, 1981) and the amount of LH released in response to GnRH also increases from the third week of lactation (Bevers *et al.*, 1981; Stevenson *et al.*, 1981; Rojansthien *et al.*, 1987). A positive-feedback response to exogenous oestradiol is also re-established by day 35 of lactation, having been absent on day 5 and greatly reduced on day 15 of lactation (Elsaesser and Parvizi, 1980; Ramirez *et al.*, 1985; Cox *et al.*, 1988; De-Rensis *et al.*, 1991), although oestradiol will induce oestrus behaviour throughout lactation (Cox *et al.*, 1988).

The LH response to weaning increases with time during lactation. In early lactation (day 10) the LH response to the natural post-weaning increase in oestradiol is poor (S. Edwards and Foxcroft, 1983; Kirkwood *et al.*, 1984a,b). In contrast, a significant increase in basal levels of LH due to an increase in LH pulse frequency but not amplitude occurs after weaning from day 2 onwards (Cox and Britt, 1982b; S. Edwards and Foxcroft, 1983; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987; E.A. Newton *et al.*, 1987; Rojkittikhun *et al.*, 1991), and is related to a significant increase in both pituitary content of LH (Crighton and Lamming, 1969; Cox and Britt, 1982b) and hypothalamic content of GnRH (Cox and Britt, 1982b). The pattern of the pulsatile secretion of LH is very variable in the later part of lactation and, while sows exhibiting early oestrus after weaning tend to have increased pulsatile LH release before weaning (Shaw and Foxcroft, 1985), there does not appear to be any relationship between post-weaning LH pulsatility and time to oestrous onset (Foxcroft *et al.*, 1987).

(c) Sheep

In the ewe the hypothalamic content of GnRH (Moss *et al.*, 1980; Crowder *et al.*, 1982; Clarke *et al.*, 1984), pituitary content of LH (Chamley *et al.*, 1976; Jenkin *et al.*, 1977; Restall and Starr, 1977; Restall *et al.*, 1977; Moss *et al.*, 1980; Crowder *et al.*, 1982; Clarke *et al.*, 1984; Wise *et al.*, 1985) and plasma levels of LH (Jenkin *et al.*, 1977; Clarke *et al.*, 1984; Gregg *et al.*, 1986; Sharpe *et al.*, 1986;

G.S. Lewis and Bolt, 1987; Malven and Hudgens, 1987) return to normal within 14 days after parturition whether or not lactation occurs, as does the mRNA content for the gonadotrophin α and LHb subunits (Wise *et al.*, 1985), GnRH receptor (Crowder *et al.*, 1982) and oestrogen receptor (Wise *et al.*, 1986). Basal levels of LH increase in parallel with an increase in pulsatile LH secretion during lactation, although the rate of increase in LH pulse frequency slows as the number of lambs suckled increases (Schirar *et al.*, 1990; Wise, 1990). The increase in pulsatile release of LH is directly related to an increase in pulsatile release of GnRH measured in the hypophyseal portal vessels (Wise, 1990). In early lactation, when pituitary LH content is low, only 30–40% of GnRH pulses release LH compared with 70–80% later in lactation (Wise, 1990). However, the effect of lactation on the resumption of oestrous cycles varies considerably among breeds of sheep even though most parameters of LH secretion appear to return to normal early in lactation (see previous section). In lactating ewes lambing during the breeding season a paradoxical increase in both basal levels of LH (Restall and Starr, 1977) and the pulsatile secretion of LH occurred (P.J. Wright *et al.*, 1981a,b,c; Clarke *et al.*, 1984), the frequency of pulses being greater than during either the luteal phase or seasonal anoestrus. The LH response to GnRH returns to normal earlier in ewes lambing in the autumn (P.J. Wright *et al.*, 1980) than those lambing in the spring during the late breeding or early anoestrous season (Chamley *et al.*, 1974; Jenkin *et al.*, 1977) but, in spite of this normal response to GnRH, only around 50% of ewes show a normal positive-feedback response to oestradiol by 30 days post-partum (P.J. Wright *et al.*, 1980, 1981a,b). Chronic treatment of lactating ewes with low-dose oestradiol decreases the LH response to GnRH (Leakakos *et al.*, 1987) and causes a greater suppression of LH in lactating than non-lactating ovariectomized ewes. These results suggest that suckling can induce an increase in sensitivity to the negative-feedback effects of oestradiol, but the response varies considerably between different breeds of ewe.

(d) Cow

Both the pituitary content of LH (Labhetswar *et al.*, 1964; Saididdin *et al.*, 1968; Cermak *et al.*, 1983; Moss *et al.*, 1985; Leung *et al.*, 1986) and the plasma concentration of LH increase to normal levels by 30–50 days post-partum (Echternkamp and Hansel, 1973; Dickery *et al.*, 1975; Goodale *et al.*, 1978; Schams *et al.*, 1978; Rawlings *et al.*, 1980; Troxel *et al.*, 1980; Webb *et al.*, 1980; Kesler *et al.*, 1981; Fisher *et al.*, 1986; I.A. Wright *et al.*, 1987; M.G. Murphy *et al.*, 1990; Savio *et al.*, 1990a; Schemm *et al.*, 1990; Spicer *et al.*, 1990; Canfield and Butler, 1991; Lucy *et al.*, 1991). The increase in basal levels results from a resumption of the pulsatile release of LH (Stevenson and Britt, 1979; Carruthers and Hafs, 1980; Carruthers et al, 1980; Forrest *et al.*, 1980; Rahe *et al.*, 1980; Lamming *et al.*, 1981; A.R. Peters *et al.*, 1981; Ramirez-Godinez *et al.*, 1982; Walters *et al.*, 1982; Fisher *et al.*, 1986; G.L. Williams *et al.*, 1987; I.A. Wright *et al.*, 1987; M.G. Murphy *et al.*, 1990; Schemm *et al.*, 1990; Lucy *et al.*, 1991; Savio *et al.*, 1990a; Canfield and Butler, 1991) and occurs more rapidly in milked than suckled cows (A.R. Peters and Lamming, 1984). The LH response to GnRH both *in vivo* (Webb *et al.*, 1977; Schallenberger *et al.*, 1978, 1982; Fernandes *et al.*, 1978; Foster *et al.*, 1980; Jaeger *et al.*, 1987) and from the pituitary *in vitro* (Moss *et al.*, 1985; Leung *et al.*, 1986) also increases with time post-partum, although there is no change in either the affinity or number of GnRH receptors in the pituitary during lactation (Moss *et al.*, 1985; H.C. Leung *et al.*, 1986). Weaning does not affect GnRH release from the stalk median eminence *in vitro* (Connor *et al.*, 1990) and hypothalamic GnRH content is greater in suckled

than non-suckled ovariectomized cows (Zalesky *et al.*, 1990).

A major increase in the pulsatile secretion of LH occurs in the few days preceding first ovulation post-partum (Figure 11.4; Walters *et al.*, 1982; P.J. Hansen and Hauser, 1983; Garcia-Winder *et al.*, 1984; A.R. Peters and Lamming, 1984; Fisher *et al.*, 1986; M.G. Murphy *et al.*, 1990; Perry *et al.*, 1991a), and this increase in LH stimulates follicle development and an increase in plasma concentrations of oestradiol (Fisher *et al.*, 1986; M.G. Murphy *et al.*, 1990). Suckling delays this increase in LH pulsatility and weaning is associated with a rapid increase in LH pulse frequency without a change in pulse amplitude (Fogwell *et al.*, 1986; Faltys *et al.*, 1987; Rutter and Manns, 1987; I.A. Wright *et al.*, 1987). This increase can be prevented by maintaining cows in a state of hypoglycaemia or negative energy balance (Rutter and Manns, 1987; Canfield and Butler, 1991; Randel, 1990; Schrick *et al.*, 1990; G.L. Williams, 1990; Lucy *et al.*, 1991) and the increase in frequency is less in cows in poor body condition (I.A. Wright *et al.*, 1987) and can be stopped by reintroduction of the suckling calf (I.A. Wright *et al.*, 1987). Increasing dietary energy can reduce the time to first ovulation, apparently by increasing LH pulse amplitude but not frequency (Canfield and Butler, 1991; 1990; Lucy *et al.*, 1991).

Oestrogen-induced positive-feedback release of LH occurs in most suckled and milked cows by day 15 post-partum (Radford *et al.*, 1978; R.E. Short *et al.*, 1979; Schallenberger *et al.*, 1982; A.R. Peters, 1984), but the amount of LH is significantly reduced compared with non-lactating cows, suggesting a suckling-induced reduction of response. The LH response to ovariectomy on days 4–5 post-partum is delayed for 5–10 days in cows suckling one or two calves (Schallenberger *et al.*, 1982; Acosta *et al.*, 1983; Garcia-Winder *et al.*, 1984; Hinshelwood *et al.*, 1985), but both plasma levels and the pulse frequency of LH increase throughout the post-partum period until they are equivalent to non-suckled ovariectomized cows by 40–60 days post-partum (Garcia-Winder *et al.*, 1984). However, this escape from the inhibitory effects of suckling alone can be delayed by treatment with oestradiol (Acosta *et al.*, 1983; Hinshelwood *et al.*, 1985). Indeed, the post-partum interval in intact suckled cows can be shortened by treatment with an anti-oestrogen (Chang and Reeves, 1987), supporting the view that suckling in the cow causes an increase in sensitivity to the negative-feedback effects of oestradiol.

(e) Non-human primates

During lactation in the rhesus monkey, plasma levels of LH remain suppressed for the first 9 months post-partum and then increase to normal follicular plasma levels by 12 months (T.M. Plant *et al.*, 1980). Injection of GnRH in the first month or of oestradiol for the first 3–6 months post-partum fails to induce an increase in LH (Yamaji *et al.*, 1971; R.F. Williams *et al.*, 1979; T.M. Plant *et al.*, 1980; Schallenberger *et al.*, 1981; Ash *et al.*, 1984; Richardson *et al.*, 1985), in contrast to non-suckled monkeys, in which a normal response returns within the first month post-partum (T.M. Plant *et al.*, 1980). The positive-feedback effect of oestrogen on LH gradually increases after 6 months to reach normal by 10–11 months post-partum (Figure 11.5; T.M. Plant *et al.*, 1980; Schallenberger *et al.*, 1981).

The suppression of gonadotrophin secretion and positive-feedback effects of oestrogen can be induced in non-post-partum rhesus monkeys fostering neonates (T.M. Plant *et al.*, 1980), confirming that the effect of lactation is due to the suckling stimulus alone and not any events associated with pregnancy or parturition (Schallenberger *et al.*, 1981). Indeed, suckling delays the increase in both LH and FSH after ovariectomy in early lactation in cynomolgus monkeys (Gordon *et al.*, 1992).

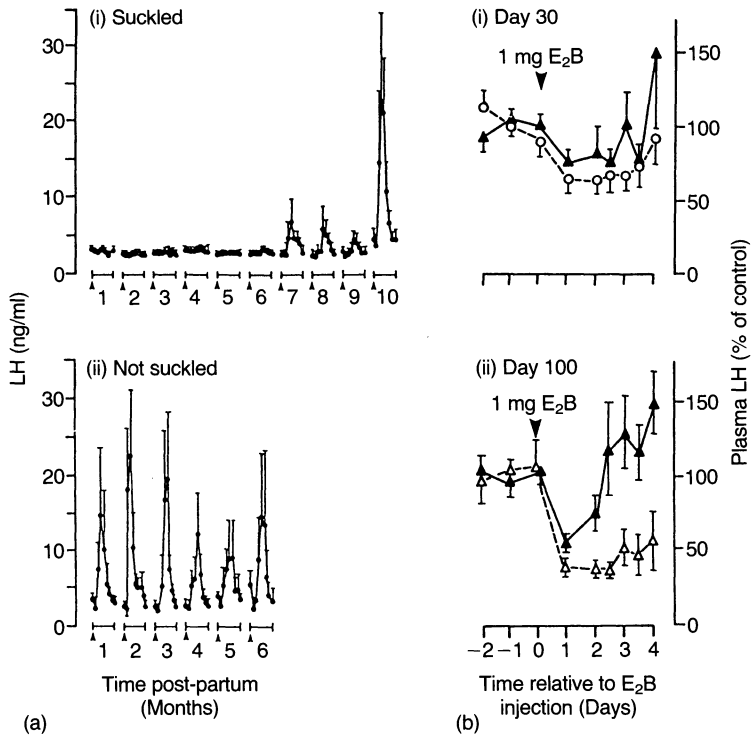

Figure 11.5 Changes in the positive- and negative- feedback effects of oestrogen on the secretion of LH with time post-partum in the rhesus monkey (A) and human (B). Note that in both suckled monkeys and humans the positive-feedback effect of oestrogen is inhibited by suckling while the negative-feedback effects of oestrogen are enhanced in humans. (From Baird *et al.*, 1979; T.M. Plant *et al.*, 1980.)

(g) Human

In non-breastfeeding humans, plasma levels of LH return to normal cycle levels within 20–25 days post-partum (Bonnar *et al.*, 1975; Rolland *et al.*, 1975; Baird *et al.*, 1979; A.S. McNeilly, 1979; Howie and McNeilly, 1982; Glasier *et al.*, 1983), while in breastfeeding humans, plasma levels increase to the lower limit of normal by day 20 and remain suppressed throughout most of the period of lactational amenorrhoea (Reyes *et al.*, 1972; Bonnar *et al.*, 1975; Rolland *et al.*, 1975; Keye and Jaffe, 1976; Delvoye *et al.*, 1978; Baird *et al.*, 1979; Duchen and McNeilly, 1980; A.S. McNeilly *et al.*, 1980; Glasier *et al.*, 1983, 1984b; Kremer *et al.*, 1991; Nunley *et al.*, 1991; Fink *et al.*, 1992; Tay *et al.*, 1992, 1993). These reduced plasma levels of LH are associated with either absent or low-frequency, low-amplitude pulses of LH (Figure 11.4; Madden *et al.*, 1978; Tyson *et al.*, 1978; Glasier *et al.*, 1984b, Gross and Eastman, 1985; Nunley *et al.*, 1991; Tay *et al.*, 1992, 1993) and a reduced LH response to large-bolus injections of exogenous GnRH (Jeppson *et al.*, 1974; Le Maire *et al.*, 1974; Andreassen and Tyson, 1976). Normal pulsatile release of LH can be induced by the injection of small boluses of GnRH (Glasier *et al.*, 1986), suggesting that there is a reduction in the total amount of LH in the pituitary but that the amount is still sufficient to allow the normal pulsatile release of LH if GnRH was secreted by the hypothalamus. During full breastfeeding, in which LH levels are suppressed, oestradiol

fails to induce a positive-feedback release of LH (Baird *et al.*, 1979; Glass *et al.*, 1981; Vermer and Rolland, 1982) and levels appear to be more suppressed than normal by oestradiol, suggesting a suckling-induced increase in sensitivity to the negative-feedback effects of oestradiol (Figure 11.5; Baird *et al.*, 1979). As breast feeding declines, plasma levels and the pulsatile secretion of LH increase, stimulating follicular oestrogen secretion and ovulation (Figure 11.4; Rolland *et al.*, 1975; P.G. Lunn *et al.*, 1980; A.S. McNeilly *et al.*, 1980; Glasier *et al.*, 1983, 1984b).

(g) Summary

The suckling or milking stimulus in all species for which there are adequate data results in a suppression of the normal pulsatile secretion of LH, with a consequent maintenance of plasma levels of LH which are lower than those in the normal oestrous or menstrual cycle. This reduction in LH is related, at least in some species, to a decrease in pituitary content of LH, a reduced GnRH receptor content and a decrease in the ability of both GnRH and oestradiol to induce a normal release of LH. These results suggest that suckling or milking suppresses the hypothalamic output of GnRH or disrupts the normal regular pattern of pulsatile release of GnRH from the hypothalamus. In contrast to LH, plasma levels of FSH usually return to normal early in post-partum lactating animals, suggesting that suckling or milking has only a minimal effect on FSH secretion. However, this is probably not the case, since both the FSH and LH response to ovariectomy is suppressed if oestradiol replacement is given, and enhanced sensitivity to the negative-feedback effects of oestradiol can be demonstrated for both FSH and LH.

11.6 INDUCTION OF OVARIAN ACTIVITY DURING LACTATION

If the lack of ovarian activity during lactation is due to the reduction in gonadotrophin secretion rather than any change in the sensitivity of the ovary to the available gonadotrophins, it should be possible to induce follicle growth and ovulation by increasing gonadotrophin secretion in the animal using appropriate GnRH stimulation or by giving exogenous gonadotrophins. In early lactation in the rat no follicles are present which are capable of ovulating in response to a single large injection of human chorionic gonadotrophin (hCG), but responsive follicles could be induced by pretreatment for 5 days with low doses of either hCG or LH (Taya and Greenwald, 1982; Taya and Sasamoto, 1987, 1988a, 1990). An increase in FSH alone occurring after immunoneutralization of GnRH in lactating rats does not affect follicle growth (Taya and Sasamoto, 1988b). In the pig, treatment with FSH or pregnant mare serum gonadotrophin (PMSG) alone or in combination with human chorionic gonadotrophin (hCG) fails to induce oestrus and ovulation early in lactation, but the percentage of sows showing oestrus and conceiving increases after the third week of lactation (Hausler *et al.*, 1980; Britt *et al.*, 1985). While continuous infusion of GnRH failed to induce oestrus (Britt *et al.*, 1985), the pulsatile administration of GnRH for 60 or more hours will induce oestrus in most sows from day 24 of lactation onwards (Cox and Britt, 1982b; Britt *et al.*, 1985) and the percentage responding decreases the earlier the treatment is started in lactation.

In both the sheep (P.J. Wright *et al.*, 1983, 1984; G.S. Lewis and Bolt, 1987) and cow (Rahe *et al.*, 1980; Riley *et al.*, 1981; A.R. Peters *et al.*, 1985; Vostermans and Walton, 1985; Spicer *et al.*, 1986a–d), ovulation can be induced by repeated injections of low doses of GnRH usually administered at a constant interval of 1–2 h, a frequency similar to that occurring in the normal follicular phase of the

cycle (Baird and McNeilly, 1981). However, the results of such treatments are variable, and while ovulation may be induced corpus luteum function is often inadequate (Garcia-Winder *et al.*, 1986; Spicer *et al.*, 1986b; Jagger *et al.*, 1987; V.G. Smith *et al.*, 1987; Braden *et al.*, 1989), a situation similar to that after treatment of lactating cows with single intramuscular injections of GnRH (Schams *et al.*, 1973; Britt *et al.*, 1974; Webb *et al.*, 1977; Kesler *et al.*, 1978, 1981; Zaied *et al.*, 1980; C.N. Lee *et al.*, 1983). In sheep, adequate luteal function after low-dose GnRH treatment occurs if the frequency of the pulsatile administration is progressively increased (3-hourly to hourly) and is further improved if ewes are pretreated with progesterone (P.J. Wright *et al.*, 1984), a situation similar to induction of ovulation in acyclic non-lactating anoestrous ewes (McLeod *et al.*, 1982; A.S. McNeilly *et al.*, 1982c; McNatty *et al.*, 1984). A similar improvement in luteal function occurs if milked or suckled cows are pretreated with progesterone before the start of GnRH treatment (Garcia-Winder *et al.*, 1986, 1987; Spicer *et al.*, 1986a,c; V.G. Smith *et al.*, 1987).

In humans, gonadotrophin stimulation immediately post-partum fails to stimulate oestrogen secretion (Zarate *et al.*, 1972; A.S. McNeilly *et al.*, 1980; Howie *et al.*, 1981), but thereafter either exogenous gonadotrophins (Nakano *et al.*, 1975; Andreassen and Tyson, 1976) or GnRH-induced increases in endogenous gonadotrophins (Zanartu *et al.*, 1974; Andreassen and Tyson, 1976; Glasier *et al.*, 1986) will stimulate follicular development, oestrogen secretion and ovulation, although as in the cow corpus luteum function may be inadequate (Glasier *et al.*, 1986).

These results show that the failure of ovarian follicular development during lactation is due principally to a lack of adequate gonadotrophin stimulation, mainly LH. Injections of a large bolus of hCG, LH or GnRH to induce a large release of endogenous LH, all of which expose the ovary to LH in amounts similar to the preovulatory LH surge, will only induce ovulation with the formation of an adequate corpus luteum if a follicle(s) is present in the ovary at a sufficiently advanced stage of development to respond adequately. Such follicles can be induced by treatment with pulsatile low-dose injections of GnRH at a frequency which mimics closely the normal increase in LH pulse frequency seen in the normal follicular phase of the oestrous or menstrual cycles. In the majority of cases this will result in ovulation and the formation of an adequate corpus luteum, and the percentage showing normal responses can be further improved by pretreatment with progesterone, although the mechanism remains unclear. The final question that remains is: how does suckling or milking suppress endogenous GnRH and hence LH secretion during lactation?

11.7 MECHANISMS OF SUCKLING/MILKING-INDUCED SUPPRESSION OF GnRH

11.7.1 PROLACTIN

Because of the close association between raised prolactin levels and the suppression of ovarian activity, it has been suggested that prolactin may play an important role in either or both the suppression of gonadotrophin secretion or inhibition of gonadotrophin action at the ovary (for discussion see A.S. McNeilly, 1979, 1984, 1985, 1987a,b, 1993; A.S. McNeilly *et al.*, 1982a). The importance of prolactin in normal ovarian function varies between species and appears to play a key role in the rat, but has less or no importance in other species (A.S. McNeilly, 1984). Thus, the role of prolactin in controlling ovarian activity will first be discussed in the rat.

The corpora lutea of lactation in the rat are dependent on prolactin, and treatment with CB154 to suppress prolactin in early and mid-lactation results in a rapid decline in plasma levels of progesterone (Tomogane *et al.*, 1969; Lu *et al.*, 1976b; Rothchild, 1981; S. Hansen *et*

al., 1983). While treatment in early lactation results in oestrus around 8–10 days later, treatment in mid-lactation (days 9–13) results in a rapid return of oestrus (Tomogane *et al.*, 1969; Lu *et al.*, 1976b; S. Hansen *et al.*, 1983). These results suggest that prolactin does play an active role in suppressing oestrus, probably indirectly via progesterone, but the different responses to CB154 appear to relate more directly to the extended delay of the LH response to CB154 in early rather than mid-lactation (Lu *et al.*, 1976b). A more clear-cut effect of prolactin *per se* on LH release is seen after ovariectomy. Suppression of prolactin with CB154 after ovariectomy in early lactation resulted in an increase in plasma levels of LH to 50% of those seen in non-lactating ovariectomized females, while FSH increased to normal castration levels (M.S. Smith, 1978b, 1981). Both LH and FSH increased to normal castration levels when prolactin was suppressed by CB154 after ovariectomy in mid-lactation (M.S. Smith, 1978b, 1981). Since the effects of CB154 could be overcome by concomitant treatment with prolactin (M.S. Smith, 1978b, 1981), this implies that prolactin *per se* was having a direct effect suppressing GnRH release after ovariectomy. Indeed, prolactin causes a reduction in GnRH receptors when administered in mid-lactation (M.S. Smith and Lee, 1989). Although such a situation may occur during lactation there does not appear to be a precise inverse relationship between prolactin and LH during any 24-h period in lactation in the rat, and maximum levels of prolactin may or may not coincide with alterations in LH (van der Schoot *et al*, 1978; Sodersten and Eneroth, 1984).

In a manner similar to lactation-induced delay in implantation in the rat, the blastocyst and corpus luteum resulting from ovulation and conception occurring at the postpartum oestrus immediately after parturition in the wallaby remains in a quiescent state maintained by the suckling joey (lactational diapause, Tyndale-Biscoe *et al.*, 1986). This lactational diapause can be terminated by removal of the suckling joey (Tyndale-Biscoe, 1984) or by hypophysectomy of the mother (Hearn, 1974), suggesting that secretion of a pituitary factor is responsible for the suppression of luteal function and the resultant embryonic diapause. Treatment of wallabies with CB154 which will suppress prolactin (Tyndale-Biscoe and Hinds, 1984; Curlewis *et al.*, 1986) results in reactivation of the corpus luteum, while treatment with prolactin will prevent reactivation (Tyndale-Biscoe and Hawkins, 1977). These results suggest that prolactin released by the suckling joey is the key hormone in preventing reactivation of the corpus luteum. If this is the case, the amounts of prolactin required must be extremely small or the corpus luteum very sensitive to prolactin since there appears to be little or no change in prolactin levels during the early stages of the diapause in the wallaby (Tyndale-Biscoe and Hinds, 1984; Hinds and Tyndale-Biscoe, 1985; Curlewis *et al.*, 1986) or other marsupials.

In the pig (Kraeling *et al.*, 1982; Bevers *et al.*, 1983; Mattioli and Seren, 1985) sheep (Louw *et al.*, 1976; Kann *et al.*, 1979; Fitzgerald and Cunningham, 1981; P.J. Wright *et al.*, 1981a) and cow (Cummins *et al.*, 1977; Clemente *et al.*, 1978; Schallenberger *et al.*, 1978; G.L. Williams and Ray, 1980; Montgomery, 1982) suppression of prolactin with CB154 during lactation had little or no effect on LH secretion or the onset of oestrus in lactation. In sows there is no correlation between prolactin levels throughout lactation and plasma levels of FSH or LH (Stevenson *et al.*, 1981; Foxcroft *et al.*, 1987), and the acute suckling stimulus has no effect on endogenous levels of LH or the LH response to GnRH (Stevenson *et al.*, 1981). Similarly, in the cow, while suckling delays oestrus for considerably longer than milking, plasma prolactin levels are similar in suckled and milked cows (Carruthers and Hafs, 1980; J.F. Smith *et al.*, 1981) and in cows suckling one or two calves (Wheeler *et al.*, 1982).

In the ewe, high levels of prolactin may directly reduce ovarian oestradiol (A.S. McNeilly and Baird, 1983) and progesterone (Rhind *et al.*, 1978) secretion and reduce the positive-feedback effect of oestradiol on LH release (Kann and Denamur, 1974; Kann and Martinet, 1975). While these effects may contribute to the reduced fertility of lactating ewes following the induction of oestrus (Restall *et al.*, 1978; Rhind *et al.*, 1980), it is more likely that this is due to an inadequacy of LH secretion induced by the suckling stimulus directly (J. Pelletier and Thimonier, 1973; Kann and Martinet, 1975; Kittock *et al.*, 1983). In the goat, maintenance of the seasonal photoperiodic increase in prolactin by continual exposure to artificially long day lengths in the autumn did not affect the normal time of onset of ovarian cyclicity (Hart, 1975b).

In red deer, a delay in the resumption of oestrous cycles after seasonal anoestrus in poorly nourished hinds was associated with increased plasma levels of prolactin (Loudon *et al.*, 1983). However, this increase in prolactin was related to a three to 10-fold increase in the frequency of suckling in the poorly nourished hinds suggesting that the prolactin changes were of secondary and minor importance. Thus, in ruminants there seems little evidence to support a major role of prolactin in suppressing gonadotrophin release during lactation.

Although injections of prolactin in bonnet monkeys may reduce the acute LH response to GnRH and extend the period of lactational infertility (Maneckjee and Moudgal, 1975; Maneckjee *et al.*, 1976), in the common marmoset, ovulatory cycles and conceptions occur soon after delivery even though mothers are usually suckled by twin offspring and have high plasma levels of prolactin associated with lactation (A.S. McNeilly *et al.*, 1981).

In the rhesus monkey, the increased plasma levels of prolactin during lactation maintain the corpus luteum of pregnancy and a low output of progesterone during the first 2–3 months of lactation (Dierschke *et al.*, 1973). However, lactational infertility lasting 9–12 months extends well beyond the time when corpus luteum function ceases and plasma levels of progesterone decline to normal follicular phase levels, suggesting that any indirect role of prolactin on the corpus luteum is of little importance in maintaining infertility. Indeed, in rhesus monkeys, both basal levels of LH and FSH and the positive-feedback effects of oestradiol continue to be inhibited by suckling when prolactin secretion is suppressed by treatment with CB154 throughout lactation (Schallenberger *et al.*, 1981).

In women, a close association between increased plasma levels of prolactin (hyperprolactinaemia) and the duration of infertility during lactation has supported the possibility that prolactin may be directly involved in lactational infertility in women (Delvoye *et al.*, 1978; Duchen and McNeilly, 1980; Andersen and Schioler, 1982; Gross and Eastman, 1983; Hennart *et al.*, 1985; Wood *et al.*, 1985a,b; Stern *et al.*, 1986; Fink *et al.*, 1992). Indeed, in some but not all studies, the increase in oestradiol associated with the resumption of ovarian follicular development is negatively correlated with the plasma level of prolactin (Delvoye *et al.*, 1978; P.G. Lunn *et al.*, 1980; Andersen and Schioler, 1982; Howie and McNeilly, 1982; Gross and Eastman, 1983; Hennart *et al.*, 1985; Wood *et al.*, 1985a,b). An increased release of prolactin in response to suckling throughout the day and night associated with prolonged lactational amenorrhoea has been reported (Diaz *et al.*, 1989, 1991), although this has not been confirmed (Tay *et al.*, 1992). However, the plasma level of prolactin in women is related to the amount of breastfeeding occurring throughout a 24-h period (Delvoye *et al.*, 1978; Konner and Worthman, 1980; Howie *et al.*, 1980; Howie and McNeilly, 1982; Gross and Eastman, 1983; Wood *et al.*, 1985a,b; Stern *et al.*, 1986; Unvas-Moberg *et al.*, 1990;

Tay *et al.*, 1992) and there is no acute effect of suckling on the release of LH or FSH (A.S. McNeilly *et al.*, 1980; Dawood *et al.*, 1981; Glasier *et al.*, 1984b).

Studies *in vitro* using cultured granulosa cells from human follicles or corpora lutea (McNatty *et al.*, 1974; W.L. Edwards *et al.*, 1982; A.S. McNeilly *et al.*, 1983b; Hunter, 1984; Alila *et al.*, 1987) suggest that prolactin may or may not have a direct inhibitory effect on steroid production (see A.S. McNeilly, 1980; A.S. McNeilly *et al.*, 1982b). Similarly, drug-induced hyperprolactinaemia during the follicular phase of the menstrual cycle may block normal follicular development (L'Hermite *et al.*, 1978, 1979; Kauppila *et al.*, 1984, 1987b; Payne *et al.*, 1985; Sowa *et al.*, 1986), while an increase in prolactin in the luteal phase appears to have little or no effect on corpus luteum function (Bennink, 1979; A.S. McNeilly, 1985). A direct effect of the drugs used to manipulate prolactin levels on the secretion of LH cannot be ruled out (A.S. McNeilly, 1987a, Payne *et al.*, 1985), and thus the role of prolactin, if any, in the suppression of gonadotrophin secretion in humans during lactational amenorrhoea must be viewed with caution. Any effect would appear to be minimal (A.S. McNeilly, 1987a,b, 1993).

Any direct effect of prolactin in suppressing GnRH output from the hypothalamus was suggested to be via the short-loop feedback effect of prolactin on dopamine, in which high levels of prolactin increased dopamine turnover in the hypothalamus, specifically in the tuberoinfundibular neurones, and this resulted in an increase in dopamine release into the hypophysial portal vessels and inhibition of further prolactin release. This short-loop feedback affecting prolactin release occurs in the rat, mouse, hamster, sheep and monkey (Hokfelt and Fuxe, 1972; Herbert and Martenz, 1983; Steger *et al.*, 1985; Bartke *et al.*, 1987; Curlewis and McNeilly, 1991). Although an increase in dopamine can inhibit the secretion of LH in some species (see A.S. McNeilly, 1987b), this is not necessarily the case (Steger *et al.*, 1985; Bartke *et al.*, 1987). In lactation in the rat not only is hypothalamic dopamine turnover decreased (Selmanoff and Wise, 1981; Demarest *et al.*, 1983) associated with an increase in neuropeptide-Y (Ciofi *et al.*, 1991) and VIP expression (Gozes and Shani, 1986), the ability of prolactin to increase dopamine turnover, the short-loop feedback, is severely attenuated (Demarest *et al.*, 1983; Moore, 1987). Blockade of dopamine action by a dopamine antagonist did not affect LH release in breast-feeding humans (Tay *et al.*, 1993). Thus, it seems unlikely that an alteration in dopamine turnover, at least related to changes in prolactin, is a principal mediator of the suckling-induced inhibition of GnRH (A.S. McNeilly, 1987b).

11.7.2 OPIOIDS

Suckling may increase hypothalamic opioid tone, and this could cause the suppression of GnRH output on the one hand and the suckling-induced increase or maintenance of elevated levels of prolactin. However, results in different species and in different experiments in the same species vary considerably. Administration of the opioid receptor blocker naloxone to lactating rats (Sirinathsinghji and Martini, 1984; Selmanoff and Gregerson, 1986; Wu *et al.*, 1992), sows (Armstrong *et al.*, 1986; Barb *et al.*, 1986; Mattioli *et al.*, 1986; Armstrong *et al.*, 1988; Kraeling and Barb, 1990), ewes (Gregg *et al.*, 1986; P.G. Knight *et al.*, 1986; Leakakos *et al.*, 1987; Malven and Hudgens, 1987) and cows (Whisnant *et al.*, 1986a–c) may result in an increase in LH secretion and, where measured, a decrease in prolactin. Although in sheep this effect may be seen in both suckled and non-suckled ewes post-partum (Leakakos *et al.*, 1987), suggesting that the response is more related to the after-effects of the high levels of progesterone during pregnancy, this is clearly not the case in sows or cows, in which the

response occurs in the presence of only minimal levels of ovarian steroids. However, in pigs (Armstrong *et al.*, 1990; Barb *et al.*, 1991), dairy cows (Canfield and Butler, 1991), rhesus monkeys (Gordon *et al.*, 1992) and humans (Kremer *et al.*, 1991; Tay *et al.*, 1993) naloxone or naltrexone treatment did not affect pulsatile LH release, and there was a minimal effect in suckled cows (Myers *et al.*, 1989; Rund *et al.*, 1989).

An increase in the release of β-endorphin into the hypophysial portal vessels in response to the suckling stimulus occurs. Although no changes in hypothalamic β-endorphin levels occur during lactation in rats (Dondi *et al.*, 1991), an increase in β-endorphin was observed in suckled cows (Connor *et al.*, 1990) and intracerebroventricular administration of β-endorphin increased prolactin secretion in the lactating rat (Taya and Sasamoto, 1989). Thus, it is possible that an alteration or increase in opioid tone within the hypothalamus may be involved in the suckling-induced maintenance of prolactin secretion and inhibition of GnRH/LH release, but the evidence at present is contradictory.

11.8 CONCLUSIONS

The suckling or milking stimulus and the metabolic changes related to milk production during lactation result in a major change in the endocrinology of the mother. In the majority of mammalian species, suckling or milking induces the release of prolactin, which is essential for milk production, with the exception of the ruminants. In this species growth hormone is of much greater importance than prolactin in maintaining milk production and growth hormone release occurs principally in response to the relative hypoglycaemia associated with the production of milk and little is released directly in response to teat or nipple stimulation. The secretion of the other metabolic hormones such as insulin, glucagon and the thyroid hormones is also modified to accommodate the metabolic load of lactation, greater changes occurring as the amount of milk produced increases, in particular in the dairy cow. While the changes in these metabolic hormones occur in response to the altered metabolism of the mother due to milk production, prolactin and oxytocin are released in response to nipple or teat stimulation. Oxytocin release in some species may become conditioned to stimuli occurring before direct stimulation, but such a conditioned release does not occur for prolactin.

In the majority of species, suckling directly inhibits the release of GnRH from the hypothalamus resulting, principally, in the inhibition of the pulsatile release of LH and the resultant inhibition of ovarian follicle growth or low steroid secretion by follicles that do grow under the influence of FSH. Plasma levels of FSH are usually normal throughout lactation. The ease of induction of ovulation during lactation suggests that there is no ovarian insensitivity to gonadotrophins during lactation and any role of prolactin in suppressing gonadotrophin secretion appears to be species dependent but very minimal. Although a suckling-induced increase in opioid tone within the hypothalamus may be involved in the maintenance of prolactin secretion, there is little evidence that opiates are involved in the suppression or disruption of the pulsatile pattern of release of GnRH during suckling. Indeed, while prolactin release must be maintained to allow continual milk production throughout lactation in most species, the influence of suckling on GnRH and hence gonadotrophin release is very variable. The exact pathways involved in directing the suckling stimulus to cause oxytocin release, prolactin release and modulation of GnRH release remain unresolved. It is clear that the suckling infant plays the only important role in controlling the endocrine changes during lactation. By varying its demand for milk it alters the metabolic–endocrine response for the required milk pro-

duction in the mother, it releases prolactin to allow this milk production and oxytocin to eject the milk and finally inhibits the release of gonadotrophins, thus preventing the resumption of ovarian activity, and hence fertility, pregnancy and the birth of the next infant, until it is ready and able to survive without total reliance on milk.

REFERENCES

Acosta, B., Tarnavosky, G.K., Platt, T.E., Hamernik, D.L., Brown, J.L., Schoernomann, H.M. and Reeves, J.J. (1983) Nursing enhances the negative effect of estrogen on LH release in the cow. *Journal of Animal Science*, **57**, 1530–1536.

Afele, S., Bryant-Greenwood, G.D., Chamley, W. and Dax, E.M. (1979) Plasma relaxin immunoreactivity in the pig at parturition and during nuzzling and suckling. *Journal of Reproduction and Fertility*, **56**, 451–457.

Aherne, F.X., Christopherson, R.J., Thompson, J.R. and Hardin, R.T. (1976) Factors affecting the onset of puberty, post-weaning estrus and blood hormone levels of lacombe gilts. *Canadian Journal of Animal Science*, **56**, 681–692.

Aiyer, M.S., Chiappa, S.A. and Fink, G. (1974) A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. *Journal of Endocrinology*, **62**, 573–588.

Akers, R.M. (1985) Lactogenic hormones binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. *Journal Dairy Science*, **68**, 501–519.

Akers, R.M., Bauman, D.E., Capuco, A.V., Goodman, G.T. and Tucker, H.A. (1981) Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. *Endocrinology*, **109**, 23–30.

Alila, H.W., Rogo, K.O. and Gombe, S. (1987) Effects of prolactin on steroidogenesis by human luteal cells in culture. *Fertility and Sterility*, **47**, 947–955.

Amenomori, Y., Chen, C.L. and Meites, J. (1970) Serum prolactin levels in rats during different reproductive states. *Endocrinology*, **86**, 506–510.

Amico, J.A. and Finley, B.E. (1986) Breast stimulation in cycling women, pregnant women and a woman with induced lactation pattern of release of oxytocin, prolactin and luteinizing hormone. *Clinical Endocrinology*, **25**, 97–106.

Andersen, A.N. and Schioler, V. (1982) Influence of breastfeeding pattern on pituitary–ovarian axis of women in an industrialized community. *American Journal of Obstetrics and Gynacology*, **143**, 673–677.

Andreassen, B. and Tyson, J.E. (1976) Role of the hypothalamic–pituitary–ovarian axis in puerperal infertility. *Journal of Clinical Endocrinology and Metabolism*, **42**, 1114–1122.

Aono, T., Aki, T., Koike, K. and Kurachi, K. (1982) Effect of Sulpiride on poor lactation. *American Journal of Obstetrics and Gynecology*, **143**, 927–932.

Armstrong, J.D., Britt, J.M. and Kraeling, R.R. (1986) Endogenous opioid peptides mediate the effects of suckling on LH during lactation in sows. *Journal of Animal Science*, **63**, Suppl. 1, 57.

Armstrong, J.D., Kraeling, R.R. and Britt, J.H. (1988) Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. *Journal of Reproduction and Fertility*, **83**, 301–308.

Armstrong, J.D., Esbenshade, K.L., Coffey, M.T., Heimer, E. and Campbell, R. (1990) Opioid control of growth hormone in the suckled sow is primarily mediated through growth hormone releasing factor. *Domestic Animal Endocrinology*, **7**, 191–198.

Ash, R.W. and Heap, R.B. (1975) Oestrogen, progesterone and corticosteroid concentration in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. *Journal of Endocrinology*, **64**, 141–154.

Ash, R.M., Koloulas, I.B., Smith, G., Eddy, C.A. and Balmaceda, J.P. (1984) Pituitary responsiveness to LH-RH during gestation and puerperium in the rhesus monkey. *Acta Europaea Fertilitatis*, **15**, 151–23.

Aso, T. and Williams, R.F. (1985) Lactational amenorrhoea in monkeys effects of suckling on prolactin secretion. *Endocrinology*, **117**, 1727–1734.

Athanasiou, V.N. and Phillips, R.W. (1978) Effect of fasting on plasma metabolites and hormones in lactating dairy cows. *American Journal of Veterinary Research*, **39**, 957–961.

Averill, R.L.W., Grattan, D.R. and Norris, S.K. (1991) Posterior pituitary lobectomy chronically attenuates the nocturnal surge of prolactin in early pregnancy. *Endocrinology*, **128**, 705–709.

Baile, C.A., Della-Fera, M.A. and Buonomo, F.C. (1986) Control and manipulation of animal growth, in *The Neurophysiological Control of Growth*, (eds P.J. Buttery, D.B. Lindsay and N.B. Haynes), Butterworths, London, pp. 105–118.

Baird, D.T. and McNeilly, A.S. (1981) Gonadotrophic control of follicular development and secretion in the sheep oestrous cycle. *Journal of Reproduction and Fertility*, Suppl. 30, 119–133.

Baird, D.T., McNeilly, A.S., Sawers, R.S. and Sharpe, R.M. (1979) Failure of oestrogen induced dishcarge of luteinizing hormone in lactating women. *Journal of Clinical Endocrinology and Metabolism*, **49**, 500–509.

Baldocchi, R.A., Tan, L. and Nicol, C.S. (1992) Processing of rat prolactin by rat tissue explants and serum *in vitro*. *Endocrinology*, **130**, 1653–1659.

Barb, C.R., Kraeling, G.B., Rampacek, G.B. and Whisnant, (1986) Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. *Biology of Reproduction*, **35**, 368–371.

Barb, C.R., Estienne, M.J., Kraeling, R.R., Marple, D.N., Rampacek, G.B., Rahe, C.H. and Sartin, J.L. (1991) Endocrine changes in sows exposed to elevated ambient temperature during lactation. *Domestic Animal Endocrinology*, **8**, 117–127.

Barnes, M.A., Kazmer, G.W., Akers, R.M. and Pearson, R.E. (1985) Influence of selection for milk yield on endogenous hormones and metabolites in Holstein heifers and cows. *Journal of Animal Science*, **60**, 271–284.

Barowicz, T. (1979) Changes of blood catecholamine levels in the sheep during machine milking. *Journal of Dairy Research*, **46**, 555–562.

Bartke, A., Morgan, W.W., Clayton, R.N., Banerji, T.K., Brodie, A.M., Parkening, T.A. and Colling, T.J. (1987) Neuroendocrine studies in hyperprolactinaemic male mice. *Journal of Endocrinology*, **112**, 215–220.

Bauman, D.E. and Currie, W.B. (1980) Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homoestasis and homeorhesis. *Journal of Dairy Science*, **63**, 1514–1529.

Bauman, D.E., Eppard, P.J., DeGeeter, M.J. and Lanza, G.M. (1985) Responses of high-producing dairy cows to long-term treatment with pituitary somatotrophin and recombinant somatotrophin. *Journal of Dairy Science*, **68**, 1352–1359.

Bellin, M.E., Hinshelwood, M.M., Hauser, E.R. and Ax, R.L. (1984) Influence of suckling and side of corpus luteum or pregnancy on folliculogenesis in post-partum cows. *Biology of Reproduction*, **31**, 849–855.

Ben-Jonathan, N. (1985) Dopamine: a prolactin-inhibiting hormone. *Endocrine Reviews*, **6**, 564–589.

Ben-Jonathan, N. and Liu, J-W. (1992) Pituitary lactotrophs, endocrine, paracrine, juxtacrine and autocrine interactions. *Trends in Endocrinology and Metabolism*, **3**, 254–258.

Ben-Jonathan, N., Laudon, M. and Garris, P.A. (1991) Novel aspects of posterior pituitary function regulation of prolactin secretion. *Frontiers in Neuroendocrinology*, **12**, 231–77.

Bennett, J.P. and Vickery, B.H. (1970) Rats and mice, in *Reproduction and Breeding Techniques for Laboratory Animals*, (ed. E.S.E. Hafez), Lea & Febiger, Philadelphia, pp. 299–315.

Bennink, H.J. (1979) Intermittent bromocriptine treatment for the induction of ovulation in hyperprolactinaemic patients. *Fertility and Sterility*, **31**, 267–272.

Benson, G.K. and Folley, S.J. (1956) Oxytocin as a stimulator for the release of prolactin from the anterior pituitary. *Nature*, **177**, 700.

Bevers, M.M., Willemse, A.H. and Kruip Th, A.M. (1978) Plasma prolactin levels in the sow during lactation and the post weaning period as measured by radioimmunoassay. *Biology of Reproduction*, **19**, 628–634.

Bevers, M.M., Willemse, A.H. and Kruip Th, A.M. (1983) The effect of bromocriptine on luteinizing hormone levels in the lactating sow: evidence for suppressive action by prolactin and the suckling stimulus. *Acta Endocrinologica*, **104**, 261–265.

Bevers, M.M, Willemse, A.H., Kruip Th, A.M. and van de Wiel, D.F.M. (1981) Prolactin levels and the LH response to synthetic LH-RH in the lactating sow. *Animal Reproduction Science*, **4**, 155–163.

Bines, J.A. and Hart, I.C. (1977) Milk or meat, in *Blood Profiles in Animal Production*. (ed. D. Lister), British Society of Animal Production, London, pp. 87–98.

Bines, J.A. and Hart, I.C. (1982) Metabolic limits to milk production, especially roles of growth hormone and insulin. *Journal of Dairy Science*, **65**, 1375–1389.

Bissett, G.W., Clark, B.J. and Haldar, J. (1970) Blood levels of oxytocin and vasopressin during suckling in the rabbit and the problem of their independent release. *Journal of Physiology*, **206**, 711–722.

Blake, C.A. (1977) Effects of estrogen and progesterone on luteinizing hormone release in

ovariectomized rats. *Endocrinology*, **101**, 1122–1129.

Blum, J.W., Jans, F., Moses, W., Frohli, D., Zemp, M., Wanner, M., Hart, I.C., Thun, R. and Keller, U. (1985) Twenty four-hour pattern of blood hormone and metabolite concentrations in high-yielding dairy cows: effects of feeding low or high amounts of starch or crystalline fat. *Zentralblatt fur Vetermaarneduzin*, **32**, 401–418.

Bohnet, H.G., Gomez, F. and Friesen, H.G. (1977) Prolactin and estrogen binding sites in the mammary gland of the lactating and non-lactating rat. *Endocrinology*, **101**, 1111–1121.

Bonnar, J., Franklin, M., Nott, P.N. and McNeilly, A.S. (1975) Effect of breast-feeding on pituitary–ovarian function after childbirth. *British Medical Journal*, **3**, 82–84.

Braden, T.D., Sawyer, H.R. and Niswender, G.D. (1989) Functional and morphological characteristics of the first coprus luteum formed after parturition in ewes. *Journal of Reproduction and Fertility*, **86**, 525–533.

Britt, J.H., Armstrong, J.D., Cox, N.M. and Esbenshade, K.L. (1985) Control of follicular development during and after lactation in sows. *Journal of Reproduction and Fertility*, Suppl. 33, 37–54.

Britt, J.H., Kittok, R.J. and Harrison, D.S. (1974) Ovulation, estrus and endocrine responses after GnRH in early post-partum cows. *Journal of Animal Science*, **39**, 915–919.

Brown, J.B., Harrison, P. and Smith, M.A. (1985) A study of returning fertility after childbirth and during lactation by measurement of urinary oestrogen and pregnanediol excretion and cervical mucus production. *Journal of Biosocial Science*, Suppl. 9, 5–23.

Bruckmaier, R., Mayer, H. and Schams, D. (1991) Effects of α- and β-adrenergic agonists on intramammary pressure and milk flow in dairy cows. *Journal of Dairy Research*, **58**, 411–419.

Bruckmaier, R.M., Schams, D. and Blum, J.W. (1992) Aetiology of disturbed milk ejection in parturient primiparous cows. *Journal of Dairy Research*, **59**, 479–489.

Bruhn, T., Ellendorf, F., Forsling, M.L. and Poulain, D.A. (1981) Neurohypophysial hormone release during natural and induced milk ejection in the sow. *Journal of Physiology (London)*, **316**, 40–41.

Bryant, G.D. and Chamley, W.A. (1976) Changes in relaxin and prolactin immuno-reactivities in ovine plasma following suckling. *Journal of Reproduction and Fertility*, **46**, 457–459.

Bryant, G.D. and Greenwood, F.C. (1972) The concentrations of human prolactin in plasma measured by radioimmunoassay: experimental and physiological modifications, in *Lactogenic Hormones*, (eds G.E.W. Wolstenholme and J. Knight), A Ciba Foundation Symposium in memory of Professor S J Folley. Churchill Livingstone, Edinburgh, pp. 197–206.

Bryner, R.W., Garcia-Winder, M., Lewis, P.E., Inskeep, E.K. and Butcher, R.L. (1990) Changes in hormonal profiles during the estrous cycle in old lactating beef cows. *Domestic Animal Endocrinology*, **7**, 181–189.

Bulman, D.C. and Lammming, G.E. (1978) Milk progesterone levels in relation to conception, repeat breeding and factors influencing a cyclicity in dairy cows. *Journal of Reproduction and Fertility*, **54**, 447–458.

Burnol, A.F., Leturque, A., Ferre, P., Kande, J. and Gerard, J. (1986) Increased insulin sensitivity and responsiveness during lactation in rats. *American Journal of Physiology*, **251**, E537–E541.

Butler, W.R. and Smith, R.D. (1989) Interrelationships between energy balance and post-partum reproductive function in dairy cattle. *Journal of Dairy Science*, **72**, 767–783.

Canfield, R.W. and Butler, W.R. (1991) Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. *Journal of Animal Science*, **69**, 740–746.

Capuco, A.V., Keys, J.E. and Smith, J.J. (1989) Somatotrophin increases thyrozine-5[40]' monodeiodinase activity in lactating mammary tissue of the cow. *Journal of Endocrinology*, **121**, 205–211.

Carpenter, C.R. (1934) A field study of the behaviour and social relations of howling monkeys. *Comparative Psychological Monographs*, **10**, 1–68.

Carruthers, T.D. and Hafs, H.D. (1980) Suckling and four times daily milking influence on ovulation, estrus and serum luteinizing hormone, glucocorticoids and prolactin in post-partum Holsteins. *Journal of Animal Science*, **50**, 919–925.

Carruthers, T.D., Convey, E.M., Kesner, J.S., Hafs, H.D. and Cheng, K.W. (1980) The hypothalamo-pituitary gonadotropic axis of suckled and non-suckled dairy cows post-partum. *Journal of Animal Science*, **51**, 949–957.

Carter, M.L., Dierschke, D.J., Rutledge, J.J. and Hauser, E.R. (1980) Effect of gonadotropin releasing hormone and calf removal on pituitary

ovarian function and reproductive performance in post-partum beef cows. *Journal of Animal Science*, **51**, 903–909.

Casida, L.E., Graves, W.E., Hauser, E.R., Lauderdale, J.W., Reisen, J.W., Saididdin, S. and Tyler, W.J. (1968) Studies on the post-partum cow. *Bulletin of the Wisconsin Agriculture Experimental Station*, **270**, 1–54.

Cermak, D.L., Braden, T., Manns, J., Niswender, G.D.and Nett, T.M. (1983) Contents of hypothalamic GnRH, pituitary FSH and LH, and pituitary receptors for GnRH and estradiol in post-partum suckled beef cows. *Journal of Animal Science* **57**, Suppl. 1, 322 Abstract No. 462.

Chamley, W.A., Findlay, J.K., Cumming, I.A., Buckmaster, J.M., Goding, J.R. (1974) Effect of pregnancy on the LH response to synthetic gonadotropin releasing hormone in the ewe. *Endocrinology*, **94**, 291–293.

Chamley, W.A., Jonas, H.A. and Parr, R.A. (1976) Content of LH, FSH, and growth hormone in pituitaries of pregnant anestrus ewes. *Endocrinology*, **98**, 1535–1538.

Chang, C.F. and Reeves, J.J. (1987) Post-partum interval in beef cows shortened by enclomiphene. *Journal of Animal Science*, **65**, 217–223.

Chen, H.J., Meuller, G.P. and Meites, J. (1974) Effect of L-dopa and somatostatin on suckling induced release of prolactin and GH. *Endocrine Research Communications*, **1**, 283–291.

Choi, Y.J., Woo, J.H. and Han, I.K. (1992) Effects of the cimaterol (CL 263, 780) on growth and cellular metabolisms in the mammary gland of rats. *Journal Nutritional Biochemistry*, **3**, 2–7.

Cholst, I.N., Wardlaw, S.L., Newman, C.B. and Frantz, A,G. (1984) Prolactin response to breast stimulation in lactating women is not mediated by endogenous opioids. *American Journal of Obstetrics and Gynecology*, **150**, 558–561.

Ciofi, P., Fallon, J.H., Croix, D., Polak, J.M. and Tramu, G. (1991) Expression of neuropeptide Y precursor-immunoreactivity in the hypothalamic dopaminergic tubero-infundibular system during lactation in rodents. *Endocrinology*, **128**, 823–834.

Clapp, C., Martinez-Escalera, G., Morales, M.T., Shyr, S.W., Grosvenor, C.E. and Mena, F. (1985) Release of catecholamines follows suckling or electrical stimulation of mammary nerve in lactating rats. *Endocrinology*, **117**, 2498–2504.

Clapp, H. (1937) A factor in breeding efficiency of dairy cattle. *Proceedings of the American Society of Animal Production*, **37**, 259–265.

Clarke, I.J., Wright, P.J., Chamley, W.A. and Burman, K. (1984) Differences in the reproductive endocrine status of ewes in the early post-partum period and during seasonal anoestrus. *Journal of Reproduction and Fertility*, **70**, 591–597.

Clayton, R.N., Solano, A.R., Garcia-Vila, A., Dufau, M.L. and Catt, K.J. (1980) Regulation of pituitary receptors for gonadotropin-releasing hormone during the rat estrous cycle. *Endocrinology*, **107**, 699–705.

Clayton, R.N., Channabasavaiah, K., Stewart, J.M. and Catt, K.J. (1982) Hypothalamic regulation of pituitary gonadotropin-releasing hormone receptors: effects of hypothalamic lesions and a gonadotropin-releasing hormone antagonist. *Endocrinology*, **110**, 1108–1115.

Clegg, M.T. and Weaver, M. (1972) Chorionic gonadotrophin secretion during pregnancy in the chimpanzee (*Pan troglodytes*). *Proceedings of the Society for Experimental Biology and Medicine*, **139**, 1170–1174.

Clemente, P.F., Short, R.E., Staigmiller, R.B. and Bellows, R.A. (1978) Effects of precalving nutrition, early weaning, CB-154 and antiprolactin treatments on post-partum interval length in beef cows. *Journal of Animal Science*, 47 Suppl. 1 351.

Cleverley, J.D. and Folley, S.J. (1970) Blood levels of oxytocin during machine milking in cows with some observations on its half life in the circulation. *Journal of Endocrinology*, **46**, 347–361.

Cobo, E. (1974) Neuroendocrine control of milk ejection in women, in *Lactogenic Hormones, Fetal Mutrition and Lactation*, (eds J.B. Josimovich, M. Reynolds and E. Cobo), Wiley, New York, pp. 433–452.

Cobo, E., De Bernal, M.M., Gaitan, E. and Quintero, C.A. (1967) Neurohyphseal hormone release in the human. II. Experimental study during lactation. *American Journal of Obstetrics and Gynecology*, **97**, 519–529.

Coch, J.A., Fielitz, C., Brovetto, J., Cabot, H.M., Coda, H. and Frapa, A. (1968) Estimation of an oxytocin-like substance in highly purified extracts from the blood of puerperal women during suckling. *Journal of Endocrinology*, **40**, 137–144.

Coe, C.L. and Rosenblum, L.A. (1978) Annual reproductive strategy of the squirrel monkey (*Saimiri sciureus*). *Folia Primatologica*, **29**, 19–42.

Cole, D.J.A., Varley, M.A. and Hughes, P.E. (1975) Studies in sow reproduction. 2. The effect

of lactation length on the subsequent reproductive performances of the sow. *Animal Production*, **20**, 401–406.

Conaway, C.H. (1971) Ecological adaptation and mammalian reproduction. *Biology of Reproduction*, **1**, 239–247.

Concannon, P.W., Butler, W.R., Hansel, W., Knight, P.J., Hamilton, J.M. (1978) Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. *Biology of Reproduction*, **19**, 1113–1118.

Connor, H.C., Houghton, P.L., Lemenager, R.P., Malven, P.V., Parfet, J.R. and Moss, G.E. (1990) Effect of dietary energy, body condition and calf removal on pituitary gonadotropins, gonadotropin-releasing hormone (GnRH) and hypothalamic opioids in beef cows. *Domesticated Animal Endocrinology*, **7**, 403–411.

Connor, J.R. and Davis, H.N. (1980a) Post-partum estrus in Norway rats. I. Behaviour. *Biology of Reproduction*, **23**, 994–999.

Connor, J.R. and Davis, H.N. (1980b) Post-partum estrus in Norway rats. II. Physiology. *Biology of Reproduction*, **23**, 1000–1006.

Connor, J.S., Tribble, R.L., Woodward, T.L., Beverly, J.R., Sorensen Jr., A.M. and Fleeger, J.L. (1974) *Journal Animal Science*, **39**, 203. Abstract No. 256.

Cooke, I., Foley, M., Lenton, E., Preston, E., Millar, D., Jenkins, A., Obiekwe, B., McNeilly, A.S., Parsons, J. and Kennedy, G. (1976) The treatment of puerperal lactation with bromocriptine. *Postgraduate Medical Journal*, Suppl. 52, 75–80.

Coppings, R.J. and McCann, S.M. (1979) Stimulatory feedback action of estradiol in intact and ovariectomized lactating rats. *Biology of Reproduction*, **21**, 219–227.

Cowie, A.T. (1966) Anterior pituitary function in lactation, in *The Pituitary Gland*, (eds G.W. Harris and B.T. Donovan), Butterworths, London, pp. 412–443.

Cowie, A.T. (1969) Variations in the yield and composition of milk during lactation in the rabbit and the galactopoietic effect of prolactin. *Journal of Endocrinology*, **44**, 437–450.

Cowie, A.T., Hartmann, P.E. and Turvey, A. (1969) The maintenance of lactation in the rabbit after hypophysectomy. *Journal of Endocrinology*, **43**, 651–662.

Cowie, A.T., Forsyth, I.A. and Hart, I.C. (1980) *Hormonal Control of Lactation*, Springer-Verlag, Berlin.

Cox, N.M. and Britt, J.H. (1982a) Relationships between endogenous gonadotropin-releasing hormone, gonadotropins, and follicular development after weaning in sows. *Biology of Reproduction*, **27**, 70–78.

Cox, N.M. and Britt, J.H. (1982b) Pulsatile administration of gonadotrophin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus. *Biology of Reproduction*, **27**, 1126–1137.

Cox, N.M., Britt, J.H., Armstrong, W.D. and Alhusen, H.D. (1983) Effect of feeding fat and altered weaning schedule on rebreeding in primiparous sows. *Journal of Animal Science*, **56**, 21–29.

Cox, N.M., Ramirez, J.L., Matamoros, I.A. and Bennett, W.A. (1988) Estrogen induces estrus unaccompanied by a preovulatory surge in luteinizing hormone in suckled sows. *Biology of Reproduction*, **38**, 592–596.

Crighton, D.B. and Lamming, G.E. (1969) The lactational anoestrus of the sow the status of the anterior pituitary–ovarian system during lactation and after weaning. *Journal of Endocrinology*, **43**, 507–517.

Cross, B.A. (1955) Neurohormonal mechanism in emotional inhibition of milk-ejection. *Journal of Endocrinology*, **12**, 29–37.

Crowder, M.E., Gillees, P.A., Tamanini, C., Moss, G.E. and Nett, T.M. (1982) Pituitary content of gonadotropins and GnRH-receptors in pregnant, post-partum and steroid-treated OVX ewes. *Journal of Animal Science*, **54**, 1235–1242.

Culler, M.D., McArthur, N.H., Dees, W.L., Owens, R.E. and Harms, P.G. (1982a) Inhibition of the post ovariectomy depletion of hypothalamic luteinizing hormone releasing hormone (LHRH) by suckling. *Biology of Reproduction*, **26**, 633–639.

Culler, M.D., McArthur, N.H., Dees, W.L., Owens, R.E. and Marms, P.G. (1982b) Immunocytochemical evidence that suckling inhibits the postovariectomy depletion of median eminence luteinizing hormone releasing hormone (LHRH). *Neuroendocrinology*, **34**, 258–264.

Cummins, L.J., Findlay, J.K. and Lawson, R.A.S. (1977) Attempt at the use of CB-154 (ergocryptine) to reduce post-partum anoestrus in Hereford heifers. *Theriogenology*, **8**, 193–197.

Curlewis, J.D. and McNeilly, A.S. (1991) Prolactin short-loop feedback prolactin inhibition of luteinizing hormone secretion during the breeding

season and seasonal anoestrus of the ewe. *Neuroendocrinology*, **54**, 279–285.

Curlewis, J.D., White, A.S., Loudon, A.S.I. and McNeilly, A.S. (1986) Effects of lactation and season on plasma prolactin concentrations and response to bromocriptine during lactation in the Bennett's wallaby (*Macropus rufogriseus rufogriseus*). *Journal of Endocrinology*, **110**, 59–66.

Davis, A.J., Maule Walker, F.M. and Saunders, J.C. (1983) The role of prolactin in the control of the onset of copious milk secretion in the goat. *Journal of Physiology*, (London) **341**, 83P.

Davis, H.N. and Connor, J.R. (1980) Male modulation of female reproductive physiology during post-partum mating in Norway rats. *Behavioral and Neural Biology*, **29**, 128–131.

Davis, S.R., Gluckman, P.D., Hodgkinson, S.C., Farr, V.C., Breir, B.H. and Burleigh, B.D. (1989) Comparison of the effects of administration of recombinant bovine growth hormone or N-Met insulin-like growth factor-I to lactating goats. *Journal of Endocrinology*, **123**, 33–39.

Dawood, M.Y., Khan-Dawood, F.S., Wahi, R.S. and Fuchs, F. (1981) Oxytocin release and plasma anterior pituitary and gonadal hormones in women during lactation. *Journal of Clinical Endocrinology and Metabolism*, **52**, 678–683.

Dehoff, H.H., Stoner, C.S., Bazer, F.W., Collier, R.J., Kraeling, R.R. and Buonomo, F.C. (1986) Temporal changes in steroids, prolactin and growth hormone in pregnant and pseudopregnant gilts during mammogenesis and lactogenesis. *Domestic Animal Endocrinology*, **3**, 95–105.

Delvoye, P., Demaegd, M., Delogne-Desnoeck, J. and Brown, C. (1977) The influence of the frequency of nursing and of previous lactation experience on serum prolactin in lactating mothers. *Journal of Biosocial Science*, **9**, 447–451.

Delvoye, P., Badawi, M., Demaegd, M. and Robyn, C. (1978) Serum prolactin, gonadotrophins and estradiol in menstruating and amenorrhoeic women during two years of lactation. *American Journal of Obstetrics and Gynecology*, **130**, 635–640.

Delvoye, P., Delogne-Desnoeck, J. and Robyn, C. (1980) Hyperprolactinaemia during prolonged lactation: Evidence for anovulatory cycles and inadequate corpus luteum. *Clinical Endocrinology*, **13**, 243–247.

Demarest, K.T., McKay, D.W., Riegle, G.D. and Moore, K.E. (1983) Biochemical indices of tuberoinfundibular dopaminergic neuronal activity during lactation: a lack of response to prolatin. *Neuroendocrinology*, **36**, 130–137.

Denbow, C.J., Perera, K.S., Gwazdauskas, F.C., Akers, R.M., Reason, R.E. and McGilliard, M.L. (1986) Effect of season and stage of lactation on plasma insulin and glucose following glucose injection in Holstein cattle. *Journal of Dairy Science*, **69**, 211–216.

De-Rensis, F., Hunter, M.G., Grant, S.A., Lancaster, R.T. and Foxcroft, G.R. (1991) Effect of estrogen administration on endogenous and luteinizing hormone-releasing-hormone-induced luteinizing hormone secretion and follicular development in the lactating sow. *Biology of Reproduction*, **44**, 975–982.

Dickery, J.F., Henricks, D.M. and Bierly, S. (1975) Hormonal relationships in post-partum Holstein cows (abstract). *Journal of Animal Science*, **41**, 348.

Diaz, S., Serón-Ferré, M., Cárdenas, H., Schiappacasse, V., Brandeis, A. and Croxatto, H.B. (1989) Circadian variation of basal plasma prolactin, prolactin response to suckling, and length of amenorrhea in nursing women. *Journal of Clinical Endocrinology and Metabolism*, **68**, 946–955.

Diaz, S., Cardenas, H., Brandeis, A., Miranda, P., Schiappacasse, V., Salvatierra, A.M., Herreros, C., Seron-Ferré, M. and Croxatto, H.B. (1991) Early differences in the endocrine profile of long and short lactational amenorrhoea. *Journal of Clinical Endocrinology and Metabolism*, **72**, 196–201.

Dierschke, D.J., Yamaji, T., Karsch, F.J., Weick, R.F., Weiss, G. and Knobil, E. (1973) Blockade by progesterone of estrogen-induced LH and FSH release in the rhesus monkey. *Endocrinology*, **92**, 1496–1501.

Djiane, J. and Durand, P. (1977) Prolactin–progesterone antogonism in self-regulation of prolactin receptors. *Nature*, **266**, 641–643.

Dobson, H. (1978) Radioimmunoassay of FSH in the plasma of post-partum dairy cows. *Journal of Reproduction and Fertility*, **52**, 45–49.

Dondi, D., Maggi, R., Panerai, A.E., Piva, F. and Limonta, P. (1991) Hypothalamic opiatergic tone during pregnancy, parturition and lactation in the rat. *Neuroendocrinology*, **53**, 460–466.

Douglas, J.D. and Butler, T.M. (1970) Chimpanzee breeding at the 6571st Aeromedical Research laboratory. *Laboratory Animal Care*, **20**, 477–482.

Drickamer, L.C. (1974) A ten-year summary of reproductive data for free-ranging Macaca mulatta. *Folia Primatologica*, **21**, 61–80.

Duchen, M.R. and McNeilly, A.S. (1980) Hyperprolactinaemia and long-term lactational amenorrhoea. *Clinical Endocrinology*, **12**, 621–627.

Dufour, J.J. and Roy, G.L. (1985) Distribution of ovarian follicular populations in the dairy cow within 35 days after parturition. *Journal of Reproduction and Fertility*, **73**, 229–235.

Duggan, R.T., Bryant, M.J. and Cunningham, F.J. (1982) Gonadotrophin, total oestrogen and progesterone concentrations in the plasma of lactating sows with particular reference to lactational oestrus. *Journal of Reproduction and Fertility*, **64**, 303–313.

Duker, E.M. and Wuttke, W. (1985) Temporal changes of pituitary dopamine and beta-endorphin concentrations in push–pull cannula experiments: response to suckling stimulus. *Fidia Research*, **Series 1**, 560–565.

Dunlap, S.E., Kiser, T.E., Cox, N.M., Thompson, F.N., Rampacek, G.B., Benyshek, L.L. and Kraeling, R.R. (1981a) Cortisol and luteinizing hormone after adrenocorticotropic hormone administration to post-partum beef cows. *Journal of Animal Science*, **52**, 587–593.

Dunlap, S.E., Kiser, T.E., Rampacek, G.B., Kraeling, R.R. and Thompson, F.N. (1981b) Effect of suckling on cortisol, progesterone and luteinizing hormone in post-partum beef cows. *Theriogenology*, **16**, 185–193.

Dunn, T.G., Ingalls, J.E., Zimmerman, D.R. and Wiltbank, J.N. (1969) Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. *Journal of Animal Science*, **29**, 719–726.

Dyck, G.W. (1983) Postweaning changes in the reproductive tract of the sow. *Canadian Journal of Animal Science*, **63**, 571–577.

Dyck, G.W., Palmer, W.M. and Simaraks, S. (1979) Post weaning plasma concentrations of luteinizing hormone and estrogens in sows: Effect of treatment with pregnant mares serum gonadotropin or estradiol-17β plus progesterone. *Canadian Journal of Animal Science*, **59**, 159–166.

Echternkamp, S.E. and Hansel, W. (1973) Concurrent changes in bovine plasma hormone levels prior to and during the first post-partum estrous cycle. *Journal of Animal Science*, **37**, 1362–1370.

Eduvie, L.O. and Dawida, P.M. (1986) Effect of suckling on reproductive activities of Bunaji cows during the post-partum period. *Journal of Agricultural Science (UK)*, **107**, 235–238.

Edwards, S. and Foxcroft, G.R. (1983) Endocrine changes in sows weaned at two stages of lactation. *Journal of Reproduction and Fertility*, **67**, 161–172.

Edwards, W.L., Haynes, S.P. and Wilson, J.D. (1982) Lack of suppressive effect of prolactin on progesterone production by human granulosa cells *in vitro*. *Proceedings of the Australian Society for Endocrinology*, **25**, 72.

Elias, M.F., Teas, J., Johnston, J. and Bora, C. (1986) Nursing practices and lactational amenorrhoea. *Journal of Biosocial Science*, **18**, 1–10.

Ellendorf, F., Forsling, M.L. and Poulain, D.A. (1982) The milk ejection reflex in the pig. *Journal of Physiology (London)*, **333**, 577–594.

Ellerkmann, E., Nagy, G.M. and Frawley, L.S. (1991) Rapid augmentation of prolactin cell number and secretory capacity by an estrogen-induced factor released from the neurointermediate lobe. *Endocrinology*, **129**, 838–842.

Ellerkmann, E., Porter, T.E., Gyorgy, M.N. and Frawley, L.S. (1992) N-Acetylation is required for the lactotrope recruitment activity of a-melanocyte-stimulating hormone and β-endorphin. *Endocrinology*, **131**, 566–570.

Ellincott, A.R., Henricks, D.M., Gimenez, T. and Kiser, T.E. (1981) Suckling induced cortisol secretion in young beef cows. *Theriogenology*, **16**, 469–475.

Elliot, J.I., King, G.J. and Robertson, H.A. (1980) Reproductive performance of the sow subsequent to weaning piglets at birth. *Canadian Journal of Animal Science*, **60**, 65–71.

Elsaesser, F. and Parvizi, N. (1980) Partial recovery of the stimulatory oestrogen feedback action on LH release during late lactation in the pig. *Journal of Reproduction and Fertility*, **59**, 63–67.

England, B.G., Hauser, E.R. and Casida, L.E. (1973) Some effects of unilateral ovariectomy in the post-partum beef cow. *Journal of Animal Science*, **36**, 45–50.

Eppard, P.J., Bauman, D.E., Curtis, C.R., Erb, H.N., Lanza, G.M. and DeGreeter, M.J. (1987) Effect of 188-day treatment with somatotropin in health and reproductive performance of lactating dairy cows. *Journal of Dairy Science*, **70**, 582–591.

Erb, R.E., Chew, B.P., Malven, P.V., D'Amico, M.F., Zamet, C.N. anmd Colenbrander, V.F. (1980) Variables associated with peripartum traits in dairy cows. VII. Hormones, calf traits

and subsequent milk yield. *Journal of Animal Science*, **51**, 143–149.

Eriksson, M., Linden, A., Stock, S. and Uvnas-Moberg, K. (1987) Increased levels of vasoactive interstinal peptide and oxytocin during suckling in lactating dogs. *Peptides*, **8**, 411–413.

Everett, J.W. (1961) The mammalian female reproductive cycle and its controlling mechanisms, in *Sex and Internal Secretions*, Vol 1 (ed. W.C. Young), Williams & Wilkins, Baltimore, 497–555.

Ewy, Z. and Barowicz, T. (1981) Interactlon of neurohypophysial and adrenal catecholamines and oxytocin hormones in the ewe during the milk ejection reflex. *Advances in Physiological Science*, **20**, 209–223.

Fahmy, M.H., Holtmann, W.B. and Baker, R.D. (1979) Failure to recycle after weaning and weaning to oestrus interval in crossbred sows. *Animal Production*, **29**, 193–202.

Faltys, G.L., Convey, E.M., Short, R.E., Keech, C.A., Fogwell, R.L. (1987) Relationship between weaning and secretion of luteinizing hormone, cortisol and transcortin in beef cows. *Journal of Animal Science*, **64**, 1498–1505.

Fernandes, L.C., Thatcher, W.W., Wilcox, C.J. and Call, E.P. (1978) LH release in response to GnRH during the post-partum period of dairy cows. *Journal of Animal Science*, **46**, 443–448.

Findlay, J.K. and Cumming, I.A. (1976) FSH in the ewe effects of season, liveweight and plane of nutrition on plasma FSH and ovulation rate. *Biology of Reproduction*, **15**, 335–342.

Fink, A.E., Fink, G. and Wilson, H. (1992) Lactation, nutrition and fertility and the secretion of prolactin and gonadotrophins in Mopan Mayan women 1992. *Journal of Biosocial Science*, **24**, 35.

Fisher, M.W., Hale, D.H., Glencross, R.G., Hathorn, D.J., Lamming, G.E. and Peters, A.R. (1986) Secretion of luteinizing hormone and oestradiol–17β in post-partum milked and suckled cows. *British Veterinary Journal*, **142**, 569–577.

Fitzgerald, B.P. and Cunningham, F.J. (1981) Effect of removal of lambs or treatment with bromocriptine on plasma concentrations of prolactin and FSH during the post-partum period in ewes lambing at different times during the breeding season. *Journal of Reproduction and Fertility*, **61**, 141–148.

Flint, D.J. (1987) Endocrine manipulation of animal growth. *Journal of Endocrinology*, **115**, 365–367.

Flint, D.J., Clegg, R.A. and Knight, C.H. (1984) Stimulation of milk secretion with inhibition of milk ejection by corticosteroids during extended lactation in the rat. *Journal of Endocrinology*, **103**, 213–218.

Flint, D.J., Tonner, E., Beattie, J. and Panton, D. (1992) Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. *Journal of Endocrinology*, **134**, 377–383.

Fluckiger, E.W. (1978) Lactation inhibition by ergot drugs, in *Physiology of Mammary Glands*, (eds A. Yokoyama, H. Mizuna and H. Nagasawa), University Park Press, Baltimore, pp. 71–80.

Flynn, A.M., Docker, M., Brown, J.B. and Kennedy, K.I. (1991) Ultrasonographic patterns of ovarian activity during breastfeeding. *American Journal of Obstetrics and Gynecology*, **165**, 2027–2031.

Fogwell, F.L., Bartlett, B.B. and Reid, W.A. (1986) Synchronized estrus and fertility of beef cows after weaning calves for short intervals. *Journal of Animal Science*, **63**, 369–376.

Folley, S.J. and Knaggs, G.S. (1966) Milk-ejection activity (oxytocin) in the external jugular vein blood of the cow, goat and sow, in relation to stimulus of milking or suckling. *Journal of Endocrinology*, **34**, 197–214.

Folley, S.J. and Young, F.G. (1938) The effect of anterior pituitary extracts on lactation in the cow. *Proceedings of the Royal Society*, **B126**, 45–76.

Ford, J.J. and Melampy, R.M. (1973) Gonadotropin levels in lactating rats: effect of ovariectomy. *Endocrinology*, **93**, 540–547.

Ford, J.J. and Yoshinaga, K. (1975) The role of prolactin in the luteotrophic process of lactating rats. *Endocrinology*, **96**, 335–339.

Forrest, D.W., Fleeger, J.L., Long, C.R., Sorensen Jr., A.M. and Harms, P.G. (1980) Effects of exogenous prolactin on peripheral luteinizing hormone levels in ovariectomized cows. *Biology of Reproduction*, **2**, 197–201.

Forsling, M.L., Reinhardt, V. and Himmler, V. (1974) Neurohypophysial hormones and prolactin release. *Journal of Endocrinology*, **63**, 579–580.

Forsling, M.L., Taverne, M.A.M., Pervizi, N., Elsaesser, F., Smidt, D. and Ellendorf, F. (1979) Plasma oxytocin and steroid concentrations during late pregnancy, parturition and lactation in the miniature pig. *Journal of Endocrinology*, **82**, 61–69.

Forsyth, I.A. (1986) Variation among species in the endocrine control of mammary growth and function: the roles of prolactin, growth hormone and placental lactogen. *Journal of Dairy Science*, **69**, 886–903.

Forsyth, I.A. and Lee, P.D. (1993) Bromocriptine treatment of periparturient goats: long term suppression of prolactin and lack of effect on lactation. *Journal of Dairy Research*, **60**, (In Press).

Foster, J.P., Lamming, G.E. and Peters, A.R. (1980) Short-term relationship between plasma LH, FSH and progesterone concentrations in post-partum dairy cows and the effect of GnRH injection. *Journal of Reproduction and Fertility*, **59**, 321–327.

Fox, S.R. and Smith, M.S. (1984) The suppression of pulsatile luteinizing hormone secretion during lactation in the rat. *Endocrinology*, **115**, 2045–2051.

Foxcroft, G.R. and Hunter, M.G. (1985) Basic physiology of follicular motivation in the pig. *Journal of Reproduction and Fertility*, Suppl. 30, 1–19.

Foxcroft, G.R., Shaw, H.J., Hunter, M.G., Booth, P.J. and Lancaster, R.T. (1987) Relationships between luteinizing hormone, follicle stimulating hormone and prolactin secretion and ovarian follicular development in the weaned sow. *Biology of Reproduction*, **36**, 175–191.

Frantz, A.G., Kleinberg, D.L. and Neol, G.L. (1972) Studies on prolactin in man. *Recent Progress in Hormonal Research*, **28**, 527–590.

Fraser, H.M, Dewart, P.J. and Smith, S.K. (1989) Luteinizing hormone releasing hormone agonist in post-partum contraception: endocrine effect on breastfeeding mothers. *Journal of Clinical Endocrinology and Metabolism*, **69**, 996.

Frawley, L.S., Mulchahey, J.J. and Neill, J.D. (1983) Nursing induced a biphasic release of prolactin in rhesus monkeys. *Endocrinology*, **112**, 558–561.

Fuchs, A.R., Cubile, L., Dawood, M.Y. and Jorgensen, F.S. (1984) Release of oxytocin and prolactin by suckling in rabbits throughout lactation. *Endocrinology*, **114**, 462–469.

Fuchs, A.R., Ayromlooi, J. and Rasmussen, A.B. (1987) Oxytocin response to conditioned and non-conditioned stimuliu in lactating ewes. *Biology of Reproduction*, **37**, 301–305.

Fujiwara, T., Honjo, S., Imaizumi, K. and Imanichi, T. (1967) The post-partum menstruation of cynomolgus monkeys kept under laboratory conditions. *Japanese Journal of Medical Science and Biology*, **22**, 181–185.

Gabai, G., Fleet, I.R. and Forsyth, I.A. (1992) Effect of bromocriptine treatment on concentrations of prolactin in blood and whey of goats in declining lactation. *Journal of Endocrinology*, **135**, Suppl.

Gala, R.R. and Westphal, U. (1965) Corticosteroid-binding globulin in the rat possible role in the imitation of lactation. *Endocrinology*, **76**, 107–114.

Garcia-Winder, M., Imakawa, K., Day, M.L., Zalesky, D.D., Kittok, R.J. and Kinder, J.E. (1984) Effect of suckling and ovariectomy in the control of luteinizing hormone secretion during the post-partum period in beef cows. *Biology of Reproduction*, **31**, 771–778.

Garcia-Winder, M., Lewis, P.E., Deaver, D.R., Smith, V.G., Lewis, G.S. and Inskeep, E.K. (1986) Endocrine profiles associated with life span of induced corpora lutea in post-partum beef cows. *Journal of Animal Science*, **62**, 1353–1362.

Garcia-Winder, M., Lewis, P.E., Townsend, E.C. and Inskeep, E.K. (1987) Effects of normestomet on follicular development in post-partum beef cows. *Journal of Animal Science*, **64**, 1099–1109.

Gerloff, B.J., Herdt, T.H., Wells, W.W., Nachrieiner, R.F., Emery, R.S. (1986) Inositol and hepatic lipidosis. 2. Effect of inositol supplementation and time from parturition on serum insulin, thyroxine and triiodothyronine and their relationship to serum and liver lipids in dairy cows. *Journal of Animal Science*, **62**, 1693–1702.

Glasier, A., McNeilly, A.S. and Baird, D.T. (1986) Induction of ovarian activity by pulsatile infusion of LHRH in women with lactational amenorrhoea. *Clinical Endocrinology*, **24**, 243–252.

Glasier, A., McNeilly, A.S. and Howie, P.W. (1983) Fertility after childbirth: changes in serum gonadotrophin levels in bottle and breastfeeding women. *Clinical Endocrinology*, **19**, 493–501.

Glasier, A., McNeilly, A.S. and Howie, P.W. (1984a) The prolactin response to suckling. *Clinical Endocrinology*, **219**, 109–116.

Glasier, A, McNeilly, A.S. and Howie, P.W. (1984b) Pulsatile secretion of LH in relation to the resumption of ovarian activity post-partum. *Clinical Endocrinology*, **20**, 415–426.

Glass, M.R., Rudd, B.T., Lynch, S.S. and Butt, W.R. (1981) Oestrogen–gonadotrophin feedback mechanisms in the puerperium. *Clinical Endocrinology*, **14**, 257–267.

Glimm, D.R., Baracos, V.E. and Kennelly, J.J. (1990) Physiology and management: Northern and in situ hybridization analyses of the effects of somatotropin on bovine mammary gene expression. *Journal of Dairy Science*, **75**, 2687–2705.

Gomendio, M. (1989) Differences in fertility and suckling patterns between primiparous and multiparous rhesus mothers (*Macaca mulatta*). *Journal of Reproduction and Fertility*, **87**, 529–542.

Goodale, W.S., Garverick, H.A., Kesler, D.J., Bierschwal, C.J., Elmore, R.G. and Youngquist, R.S. (1978) Transitory changes of hormones in plasma of post-partum dairy cows. *Journal of Dairy Science*, **61**, 740–746.

Goodman, A.L. and Hodgen, G.D. (1978) Post-partum patterns of circulating FSH, LH, prolactin, estradiol and progesterone in non-suckling cynomolgus monkeys. *Steroids*, **31**, 731–744.

Gordon, K., Renfree, M.B., Short, R.V. and Clarke, I.J. (1987) Hypothalamo-pituitary portal blood concentration of B-endorphin during suckling in the ewe. *Journal of Reproduction and Fertility*, **79**, 397–408.

Gordon, K., Hodgen, G.D. and Richardson, D.W. (1992) Post-partum lactational anovulation in a nonhuman primate (*Macaca fascicularis*): endogenous opiate mediation of suckling-induced hyperprolactinaemia. *Journal of Clinical Endocrinology and Metabolism*, **75**, 59–67.

Gorewit, R.C. (1979) Method for determining oxytocin concentrations in unextracted sera; characterization in lactating cattle. *Proceedings of the Society for Experimental Biology and Medicine*, **160**, 80–87.

Gorewit, R.C. and Aromando, M.C. (1985) Mechanisms involved in the adrenalin-induced blockade of milk ejection in dairy cattle. *Proceedings of the Society for Experimental Biology and Medicine*, **180**, 340–347.

Gorewit, R.C., Wachs, E.A., Sagi, R. and Merrill, W.G. (1983) Current concepts on the role of oxytocin in milk ejection. *Journal of Dairy Science*, **66**, 2236–2250.

Gosden, R.G., Russell, J.A., Clarke, J., Piper, I. and McNeilly, A.S. (1981) Effects of inhibiting prolactin secretion on the maintenance of embryonic diapause in the suckling rat. *Journal of Endocrinology*, **88**, 197–203.

Gow, C.B., McDowell, G.H. and Jenkin, G. (1983) The importance of prolactin for initiation of lactation in the pregnant ewe. *Australian Journal of Biological Sciences*, **36**, 357–367.

Gozes, I. and Shani, Y. (1986) Hypothalamic vasoactive intestinal peptide messenger ribonucleic acid is increased in lactating rats. *Endocrinology*, **119**, 2497–2501.

Gray, R.H., Campbell, O.M., Zacur, H.A., Labbok, M.H. and MacRae, S.L. (1987) Post-partum return of ovarian activity in non-breastfeeding women monitored by urinary assays. *Journal of Clinical Endocrinology and Metabolism*, **64**, 645–650.

Gray, R.H., Campbell, O.M. and Apelo, R. (1990) Risk of ovulation during lactation. *Lancet*, **335**, 25–29.

Gregg, D.W., Moss, G.E., Hudgens, R.E. and Malven, P.V. (1986) Endogenous opioid modulation of luteinizing hormone and prolactin secretion in post-partum ewes and cows. *Journal of Animal Science*, **63**, 838–847.

Gross, B.A. and Eastman, C.J. (1983) Effect of breastfeeding status on prolactin secretion and resumption of menses. *Medical Journal of Australia*, **1**, 313–20.

Gross, B.A. and Eastman, C.J. (1985) Prolactin and the return of ovulation in breastfeeding women. *Journal of Bioscience*, Suppl. 9, 25–42.

Grosvenor, C.E., Shyr, S.W., Goodman, G.T. and Mena, F. (1986) Comparison of plasma profiles of oxytocin and prolactin following suckling in the rat. *Neuroendocrinology*, **43**, 679–685.

Grota, L.J. and Eik-nes, K.B. (1967) Plasma progesterone concentrations during pregnancy and lactation in the rat. *Journal of Reproduction and Fertility*, **13**, 83–91.

Guzman, V., Toscano, G., Canales, E.S. and Zarate, A. (1979) Improvement of defective lactation by using oral metoclopramide. *Acta Obstetrica Gynaecologica Scandinavica*, **58**, 53–55.

Hammons, J., Velasco, M. and Rothschild, I. (1973) Effect of the sudden withdrawal or increase of suckling on serum LH levels in ovariectomized postparturient rats. *Endocrinology*, **92**, 206–211.

Hansen, P.J. (1985) Seasonal modulation of puberty and the post-partum anestrus in cattle a review. *Livestock Production Sciences*, **12**, 309–327.

Hansen, P.J. and Hauser, E.R. (1983) Genotype × environmental interactions on reproductive traits of bovine females. III. Seasonal variation in post-partum reproduction as influenced by genotype, suckling and dietary regimen. *Journal of Animal Science*, **56** 1362–1369.

Hansen, P.J. and Hauser, E.R. (1984) Photoperiodic alteration of post-partum repro-

ductive function in suckled cows. *Theriogenology*, **22**, 1–14.

Hansen, S., Sodersten, P. and Eneroth, P. (1983) Mechanisms regulating hormone release and the duration of dioestrus in the lactating rat. *Journal of Endocrinology*, **99**, 173–180.

Hauser, S.D., McGrath, M.F., Collier, R.J. and Krivi, G.G. (1990) Cloning and *in vivo* expression of bovine growth hormone receptor mRNA. *Molecular and CellulE Endocrinology*, **72**, 187–200.

Harcourt, A.H., Stewart, K.J. and Watts, D.P. (1980) Reproduction in wild gorillas and some comparisons with the chimpanzee. *Journal of Reproduction and Fertility*, Suppl. 28, 59–70.

Hart, I.C., Chadwick, P.M.E., James, S. and Simmonds, A.D. (1985) Effect of intravenous bovine growth hormone or human pancreatic growth hormone-releasing factor on milk production and plasma hormones and metabolites in sheep. *Journal of Endocrinology*, **105**, 189–196.

Hart, I.C. (1973) Effect of 2-bromo-ergocriptine on milk yield and the level of prolactin and growth hormone in the blood of the goat at milking. *Journal of Endocrinology*, **57**, 179–180.

Hart, I.C. (1974) The relationship between lactation and the release of prolactin and growth hormone in the goat. *Journal of Reproduction and Fertility*, **39**, 485–499.

Hart, I.C. (1975a) Concentrations of prolactin in serial blood samples from goats before, during and after milking throughout lactation. *Journal of Endocrinology*, **64**, 305–312.

Hart, I.C. (1975b) Seasonal factors affecting the release of prolactin in goats in response to milking. *Journal of Endocrinology*, **64**, 313–322.

Hart, I.C. (1983) Endocrine control of nutrient partition in lactating ruminants. *Proceedings of the Nutrition Society*, **42**, 181–202.

Hart, I.C. and Flux, D.S. (1973) The release of growth hormone in response to milking in the goat during early and late lactation. *Journal of Endocrinology*, **57**, 177–178.

Hart, I.C. and Linzell, J.L. (1977) An analysis of specific stimuli causing the release of prolactin and growth hormone at milking in the goat. *Journal of Endocrinology*, **72**, 163–171.

Hart, I.C. and Morant, S.V. (1980) Roles of prolactin, growth hormone, insulin and thyroxine in steroid-induced lactation in goats. *Journal of Endocrinology*, **84**, 343–351.

Hart, I.C., Bines, J.A. and Morant, S.V. (1979) Endocrine control of energy metabolism in the cow: correlations of hormones and metabolites in high and low yielding cows for stages of lactation. *Journal of Dairy Science*, **62**, 270–277.

Hart, I.C., Bines, J.A., Morant, S.V. and Ridley, J.L. (1978) Endocrine control of energy metabolism in the cow; comparison of the levels of hormones (prolactin growth hormone, insulin and thyroxine) and metabolites in the plasma of high- and low-yielding cattle at various stages of lactation. *Journal of Endocrinology*, **77**, 333–345.

Hausler, C.L., Hodson, H.H., Kuo, D.C., Kinney, T.J., Rauwolf, V.A. and Strack, L.E. (1980) Induced ovulation and conception in lactating sows. *Journal of Animal Science*, **50**, 773–778.

Hearn, J.P. (1974) The pituitary gland and implantation in the tammar wallaby, Marcopus eugenii. *Journal of Reproduction and Fertility*, **39**, 235–241.

Henderson, R. and Hughes, P.E. (1984) The effects of partial weaning, movement and boar contact on the subsequent reproductive performance of lactating sows. *Animal Production*, **39**, 131–135.

Hennart, P., Delogne-Desnoeck, J., Vis, H. and Robyn, C. (1981) Serum levels of prolactin and milk production in women during a lactation period of thirty months. *Clinical Endocrinology*, **14**, 349–353.

Hennart, P., Hofvander, Y., Vis, H. and Robyn, C. (1985) Comparative study of nursing mothers in Africa (Zaire) and in Europe (Sweden): breastfeeding behaviour, nutritional status, lactational hyperprolactinaemia and status of the menstrual cycle. *Clinical Endocrinology*, **22**, 179–187.

Herbert, J. and Martenz, N.D. (1983) The effects of intraventricular prolactin infusions on pituitary responsiveness to thyrotropin releasing hormone, 5–hydroytryptophan or morphine in rhesus monkeys. *Brain Research*, **258**, 251–262.

Higuchi, T., Honda, K., Fukuoka, T., Negoro, H. and Wakabayashi, K. (1985) Release of oxytocin during suckling and parturition in the rat. *Journal of Endocrinology*, **105**, 339–346.

Higuchi, T., Tadokoro, Y., Honda, K. and Negoro, H. (1986) Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *Journal of Endocrinology*, **110**, 251–256.

Hinds, L.A. and Janssens, P.A. (1986) Changes in prolactin in peripheral plasma during lactation in the bushtail possum Trichosurus vulpecula. *Australian Journal of Biological Sciences*, **39**, 171–178.

Hinds, L.A. and Merchant, J.C. (1986) Plasma pro-

lactin concentrations throughout lactation in the eastern quoll, Dasyurus viverrinus (*marsupalia dasyuridase*). *Australian Journal of Biological Sciences*, **39**, 179–186.

Hinds, L.A., Tyndale-Biscoe, C.H. (1982) Prolactin in the marsupial Macropus eugenii, during the estrous cycle, pregnancy and lactation. *Biology of Reproduction*, **26**, 391–398.

Hinds, L.A., Tyndale-Biscoe, C.H. (1985) Seasonal and circadian prolactin during lactation and seasonal quiescence in the tammar, Macropus eugenii. *Journal of Reproduction and Fertility*, **74**, 173–183.

Hinshelwood, M.M., Dierschke, D.J. and Hauser, E.R. (1985) Effect of suckling on the hypothalamic–pituitary–axis in post-partum beef cows, independent of ovarian secretion. *Biology of Reproduction*, **32**, 290–300.

Hodson, C.A., Simpkins, J.W. and Meites, J. (1978) Inhibition of luteinizing hormone release and luteinizing hormone releasing hormone action by the ovaries of post-partum lactating rats. *Endocrinology*, **102**, 832–836.

Hoffman, B., Schams, D., Grimenez, T., Ender, M.K., Herrmann, Ch. and Karg, H. (1973) Change of progesterone, total oestrogens, corticosteroids, prolactin and LH in bovine peripheral plasma around parturition with special reference to the effect of exogenous corticoids and a prolactin inhibitor respectively. *Acta Endocrinologica*, **73**, 389–395.

Hofmeyer, G.J., Van Iddekinge, B. and Blott, J.A. (1985) Domperidone secretion in breast milk and effect on puerperal prolactin levels. *British Journal of Obstetrics and Gynaecology*, **92**, 141–144.

Hokfelt, T. and Fuxe, K. (1972) Effects of prolactin and ergot alkaloids on the tuberoinfundibular dopamine (DA) neuron. *Neruoendocrinology*, **9**, 100–122.

Holst, N., Henssen, T.G., Burhol, P.G., Jorde, R., Maltau, J.M. and Haug, E. (1986) Gut peptides in lactation. *British Journal of Obstetrics and Gynaecology*, **93**, 188–193.

Hooley, R.D., Campbell, J.J. and Findlay, J.K. (1978) The importance of prolactin for lactation in the ewe. *Journal of Endocrinology*, **79**, 301–310.

Houdebine, L., Delouis, C. and Devinoy, E. (1978) Post-transcriptional stimulation of casein synthesis by thyroid hormone. *Biochemistry*, **60**, 809–812.

Howie, P.W. and McNeilly, A.S. (1982) Effect of breastfeeding patterns on human birth intervals. *Journal of Reproduction and Fertility*, **65**, 545–557.

Howie, P.W., McNeilly, A.S., McArdle, T., Smart, L. and Houston, M.J. (1980) The relationship between suckling-induced prolactin response and lactogenesis. *Journal of Clinical Endocrinology and Metabolism*, **50**, 670–673.

Howie, P.W., McNeilly, A.S., Houston, M.J., Cook, A. and Boyle, H. (1981) Effect of supplementary food on suckling patterns and ovarian activity during lactation. *British Medical Journal*, **283**, 757–759.

Howie, P.W., McNeilly, A.S., Houston, M.J., Cook, A. and Boyle, H. (1982) Fertility after childbirth: post-partum ovulation and menstruation in bottle and breastfeeding mothers. *Clinical Endocrinology*, **17**, 323–332.

Hubinont, C., Dufrane, S.P., Carcia-Morales, P., Valverde, I., Sener, A. and Malaisse, W.J. (1986) Influence of lactation upon pancreatic islet function. *Endocrinology*, **118**, 687–694.

Hunter, M.G. (1984) Prolactin stimulates steroidogenesis by human luteal tissue *in vitro*. *Journal of Endocrinology*, **103**, 107–110.

Hurnik, J.F., King, G.F. and Robertson, H.A. (1975) Estrus and related behaviour in post-partum Holstein cows. *Applied Animal Ethology*, **2**, 55–62.

Isherwood, K.M. and Cross, B.A. (1980) Effect of the suckling stimulus on secretion of prolactin and luteinizing hormone in conscious and anaesthetized rats. *Journal of Endocrinology*, **87**, 437–444.

Israngkura, B., Kennedy, K.I. and Leelapatana, B. (1989) Breastfeeding and the return to ovulation in Bangkok. *International Journal of Gynecology and Obstetrics*, **30**, 33.

Iwatani, Y., Amino, N., Tanizawa, 0., Mori, H., Kawashima, M., Yabu, Y. and Miyai, K. (1987) Decrease in free thyroium in serum of lactating women. *Clinical Chemistry*, **33**, 1217–1219.

Jacquemet, N. and Prigge, E.C. (1991) Effect of increased postmilking prolactin concentrations on lactation, plasma metabolites and pancreatic hormones in lactating goats. *Journal of Dairy Science*, **74**, 109–114.

Jaeger, J.R., Turner, M.A. and Stormshak, F. (1987) Gonadotropin releasing hormone-induced secretion of luteinizing hormone during the milk-ejection reflex in the post-partum beef cow. *Journal of Animal Science*, **65**, 543–547.

Jagger, J.P., Peters, A.R. and Lamming, G.E.

(1987) Hormone responses to low-dose GnRH treatment in post-partum beef cows. *Journal of Reproduction and Fertility*, **80**, 263–269.

Jahn, G.A., Djiane, J. and Houdebine, L.-M. (1989) Inhibition of casein synthesis by progestagens: *in vitro* modulation in relation to concentration of hormones that synergize with prolactin. *Journal of Steroid Biochemistry*, **32**, 373–379.

Jakubowski, M. and Terkel, J. (1986a) Prolactin release and milk ejection in rats suckling underfed pups. *Endocrinology*, **118**, 8–13.

Jakubowski, M. and Terkel, J. (1986b) Lactational performance, consummatory behaviour, and suppression of estrous cyclicity in rats suckling underfed pups. *Biology of Reproduction*, **35**, 119–125.

Jenkin, G., Heap, R.B. and Symons, D.B.A. (1977) Pituitary responsiveness to synthetic LHRH and pituitary LH content at various reproductive stages in the sheep. *Journal of Reproduction and Fertility*, **49**, 207–214.

Jeppson, S., Rannevik, G. and Kullander, S. (1974) Studies on the decreased gonadotropin response after administration of LH/FSH-releasing hormone during pregnancy and puerperium. *American Journal of Obstetrics and Gynecology*, **120**, 1029–1034.

Jochle, W., Ballabio, R. and di Salle, E. (1987) Inhibition of lactation in the Beagle bitch with the prolactin inhibitor cabergolinea (FCE 21336): dose response and aspects of long term safety. *Theriogenology*, **27**, 799–810.

Johnston, J.M. and Amico, J.A. (1986) A prospective longitudinal study of the release of oxytocin and prolactin in response to infant suckling in long term lactation. *Journal of Clinical Endocrinology and Metabolism*, **62**, 653–657.

Jones, R.G., Ilic, V. and Williamson, D.H. (1984) Physiological significance of altered insulin metabolism in the conscious rat during lactation. *Journal of Biochemistry*, **220**, 455–460.

Kann, G. and Denamur, R. (1974) Possible role of prolactin during the oestrous cycle and gestation in the ewe. *Journal of Reproduction and Fertility*, **39**, 473–483.

Kann, G. and Martinet, J. (1975) Prolactin levels and duration of post-partum anoestrus in lactating ewes. *Nature*, **257**, 63–64.

Kann, G., Martinet, J. and Shirar, A. (1979) Hypothalamic-pituitary control during lactation in sheep, in *Control of Ovulation*, (eds D.B. Crighton, N.B. Haynes, G.R. Foxcroft and G.E. Lamming), Butterworths, London, pp. 31–333.

Karg, H., Schams, D. and Reinhardt, V. (1972) Effects of 2-Br-α-ergocriptine on plasma prolactin levels and milk yield in cows. *Experientia*, **28**, 574–576.

Kaufman, J.C. (1965) A three-year study of mating behaviour in a free-ranging band of rhesus monkeys. *Ecology*, **46**, 500–512.

Kauppila, A., Kivinen, S. and Ylikorkala, O. (1981) A dose response relation between improved lactation and metoclopramide. *Lancet*, **i**, 1175–1177.

Kauppila, A., Kirkinen, P., Orava, M. and Vihko, R. (1984) Effects of metoclopramide-induced hyperprolactinaemia during early follicular development on human ovarian function. *Journal of Clinical Endocrinology and Metabolism*, **59**, 875–881.

Kauppila, A., Charelain, P., Kirkinen, P., Kivinen, S. and Riokonen, A. (1987a) Isolated prolactin deficiency in a women with puerperal alactogenesis. *Journal of Clinical Endocrinology and Metabolism*, **64**, 309–312.

Kauppila, A., Isotalo, H., Kirkinen, P., Makila, U.-M., Orava, M. and Vihko, R. (1987b) Metoclopramide-induced hyperprolactinaemia effects on corpus luteum function, endometrial steroid receptor concentrations and 17β–hydroxysteroid dehydrogenase activity. *Clinical Endocrinology*, **26**, 145–154.

Kazmer, G.W., Barnes, M.A. and Canfield, R.W. (1985) Reproductive and metabolic hormones during estrus after fasting in holstein heifers. *Theriogenology*, **24**, 619–629.

Kazmer, G.W., Barnes, M.A., Akers, R.M. and Pearson, R.E. (1986) Effect of genetic selection for milk yield and increased milking frequency on plasma growth hormone and prolactin concentration in holstein cows. *Journal of Animal Science*, **63**, 1220–1227.

Keech, C.A., Short, R.E. and Convey, E.M. (1983) Corticoid-binding globulin (CBG) concentrations increase in beef cows post weaning. *Journal of Animal Science*, **57**, Suppl. 1, 347.

Kemp, B. and Niall, H.D. (1984) Relaxin. *Vitamins and Hormones*, **41**, 79–115.

Kendall, J.Z., Richards, G.E. and Shih, L.-C.N. (1983) Effect of halperidol, suckling, oxytocin and hand milking on plasma relaxin and prolactin concentrations in cyclic and lactating pigs. *Journal of Reproduction and Fertility*, **69**, 271–277.

Kendrick, K.M., Keverne, E.B., Baldwin, B.A. and

Sharman, D.F. (1986) Cerebrospinal fluid levels of acetylcholinesterase, monoamines and oxytocin during labour, parturition, vaginocervical stimulation, lamb separation and suckling in sheep. *Neuroendocrinology*, **44**, 149–156.

Kennedy, K.I. and Visness, C.M. (1992) Contraceptive efficacy of lactational amenorrhoea. *Lancet*, **339**, 227.

Kennedy, K.I. Rivera, R. and McNeilly, A.S. (1989) Consensus statement on the use of breastfeeding as a family planning method. *Contraception*, **39**, 477.

Kesler, D.J., Garverick, H.A., Youngquist, R.S., Elmore, R.G. and Bierschwal, C.J. (1978) Ovarian and endocrine responses and reproductive performance following GnRH treatment in early post-partum dairy cows. *Theriogenology*, **9**, 363–369.

Kesler, D.J., Weston, P.G., Pimentel, C.A., Troxel, T.R., Vincent, D.L. and Hixon, J.E. (1981) Diminution of the *in vitro* response to luteinizing hormone by corpora lutea induced by gonadotropin releasing hormone treatment of post-partum suckled beef cows. *Journal of Animal Science*, **53**, 749–754.

Keye Jr., W.R. and Jaffe, R.B. (1976) Changing patterns of FSH and LH response to gonadotropin-releasing hormone in the puerperium. *Journal of Clinical Endocrinology and Metabolism*, **42**, 1133–1138.

Khurana, M.K. and Madan, M.L. (1986) Thyroidal hormones in relation to stage of lactation and milk yield in cattle and buffaloes. *Indian Journal of Animal Sciences*, **56**, 324–327.

King, G.J., Hurnick, J.F. and Robertson, H.A. (1976) Ovarian function and estrus in dairy cows during early lactation. *Journal of Animal Science*, **42**, 688–692.

Kirkwood, R.N., Smith, W.C. and Lapwood, K.R. (1983) Effect of partial weaning of piglets on lactational oestrus and sow and piglet body weight changes. *New Zealand Journal of Experimental Agriculture*, **11**, 231–233.

Kirkwood, R.N., Lapwood, K.R., Smith, W.C., Motler, K., Garnick, D.J. (1984a) Effects of oestradiol benzoate treatment on the reproductive performance and endocrine status of sows after lactations of 10 or 35 days. *Journal of Reproduction and Fertility*, **72**, 329–337.

Kirkwood, R.N., Lapwood, K.R., Smith, W.C. and Anderson, I.L. (1984b) Plasma concentrations of LH, prolactin, oestradiol–17β and progesterone in sows weaned after lactations of 10 or 35 days. *Journal of Reproduction and Fertility*, **70**, 95–102.

Kittock, R.J., Stellflug, J.N. and Lowry, S.R. (1983) Enhanced progesterone and pregnancy rate after gonadotropin administration in lactating ewes. *Journal of Animal Science*, **56**, 652–655.

Kleinberg, D.L., Ruan, W., Catanese, V., Newman, C.B. and Feldman, M. (1990) Nonlactogenic effects of growth hormone on growth and insulin-like growth factor-I messenger ribonucleic acid or at mammary gland. *Endocrinology*, **126**, 3274–326.

Knight, C.H., Calvert, D.T. and FLint, D.J. (1986) Inhibitory effects of bromocriptine on mammary development and function in lactating mice. *Journal of Endocrinology*, **110**, 263–270.

Knight, C.H., Foran, D. and Wilde, C.J. (1990) Interactions between autocrine and endocrine control of milk yield thrice-daily milking of bromocriptine-treated goats. *Journal of Reproduction and Fertility*, Abst. 5, 30.

Knight, P.G., Howles, C.M. and Cunningham, F.J. (1986) Evidence that opioid peptides and dopamine participate in the suckling-induced release of prolactin in the ewe. *Neuroendocrinology*, **44**, 29–35.

Konner, M. and Worthman, C. (1980) Nursing frequency, gonadal function and birth spacing among Kung hunter gatherers. *Science*, **207**, 788–791.

Koprowski, J.A. and Tucker, H.A. (1973a) Serum prolactin during various physiological states and its relationship to milk production in the bovine. *Endocrinology*, **92**, 1480–1487.

Koprowski, J.A. and Tucker, H.A. (1973b) Bovine serum growth hormone, corticoids and insulin during lactation. *Endocrinology*, **93**, 645–651.

Kraeling, R.R., Rampacek, G.B., Cox, N.M. and Kiser, T.E. (1982) Prolactin and luteinizing hormone secretion after bromocryptine (CB-154) treatment in lactating sows and ovariectomized gilts. *Journal of Animal Science*, **54**, 1212–1220.

Kraeling, R.R. and Barb, C.R. (1990) Hypothalamic control of gonadotrophin and prolactin secretion in pigs. *Journal of Reproduction and Fertility*, Supplement 40, 3–17.

Kremer, J.A., Borm, G. and Schellekens, L.A. (1991) Pulsatile secretion of luteinizing hormone and prolactin in lactating and nonlactating women and the response to naltrexone. *Journal of Clinical Endocrinology and Metabolism*, **72**, 294.

Kuanjshkekova, G.A. (1976) Reflex discharge of

catecholamines during milk ejection inhibition in goats. *Akademy Nauk SSR Biology*, **14**, 62.

Kuhn, N.J. (1983) The biochemistry of lactogenesis, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 351–353.

Kuravongkrit, A. (1984) Clinical, morphological and endocrinological studies in primiparous post-partum sows. *PhD Thesis*, Sveriges Lantbruksunivarsitet, Uppsala, Sweden.

Kuravongkrit, A., Einarsson, S., Settergren, I. (1982) Follicular developments in primiparous lactating sows. *Animal Reproduction Science*, **5**, 47–56.

Kuravongkrit, A., Edqvist, L.-E. and Einarsson, S. (1983a) Clinical and endocrinological studies in primiparous zero-weaned sows. 3. Hormonal patterns of ovarian disorders due to zero-weaning. *Zentrablatt für Veterinarmedizin Reike A*, **30**, 625–636.

Kuravongkrit, A., Kindahl, H., Einarsson, S. and Edqvist, L.-E. (1983b) Clinical and endocrinological studies in primiparous zero-weaned sows. 1. Clinical and morphological findings with special reference to the effect of PGF2 treatment. *Zentbl VetMedical*, **A30**, 607–615.

L'Hermite, M., Delogne-Desnoeck, J., Michaux-Duchene, A. and Robyn, C. (1978) Alteration of feedback mechanism of estrogen on gonadotropin by sulpiride-induced hyperprolactinaemia. *Journal of Clinical Endocrinology and Metabolism*, **47**, 1132–1136.

L'Hermite, M., Michaux-Duchene, A. and Robyn, C. (1979) Tiapride-induced chronic hyperprolactinaemia interference with the human menstrual cycle. *Acta Endocrinologica*, **92**, 214–227.

Labhsetwar, A.P., Collins, W.E., Tyler, W.J. and Casia, L.E. (1964) Some pituitary–ovarian relationships in the periparturient cow. *Journal of Reproduction and Fertility*, **8**, 85–90.

Lamming, G.E. (1966) Nutrition and the endocrine system. *Nutrition Abstracts*, **36**, 1–13.

Lamming, G.E., Moseley, S.R. McNeilly, J.R. (1974) Prolactin release in the sheep. *Journal of Reproduction and Fertility*, **40**, 151–168.

Lamming, G.E., Wathes, D.C. and Peters, A.R. (1981) Endocrine patterns of the post-partum cow. *Journal of Reproduction and Fertility*, Suppl. 30, 155–170.

van Landeghem, A.A.J. and van de Wiel, D.F.M. (1978) Radioimmunoassay for porcine prolactin plasma levels during lactation, suckling and weaning and after TRH administration. *Acta Endocrinologica*, **88**, 653–667.

Lau, C. and Henning, S.J. (1987) Mammary resistance: a possible controlling factor in milk ejection. *Journal of Endocrinology*, **112**, 379–385.

Lauderdale, J.W., Kirkpatrick, R.L., First, N.L., Hauser, E.R. and Casida, L.E. (1965) Ovarian and pituitary gland changes in periparturient sows. *Journa] of Animal Science*, **24**, 1100–1103.

Laudon, M., Grossman, D.A. and Ben-Johathan, N. (1990) Prolactin-releasing factor: cellular origin in the intermediate lobe of the pituitary. *Endocrinology*, **126**, 3185–3192.

Lawson, D.M. and Graf, G.C. (1968) Plasma oxytocic activity and intramammary pressure in lactating dairy cows. *Journal of Dairy Science*, **51**, 1676–1679.

Leakakos, T., Hudgens, R.E., Diekman, M.A. and Moss, G.E. (1987) Effect estradiol-17B, naloxone and gonadotropin releasing hormone on post-partum secretion of luteinizing hormone in fall-lambing ewes. *Journal of Animal Science*, **64**, 1484–1490.

Leake, R.D. and Fisher, D.A. (1985) Oxytocin secretion and milk ejection in the human. *Excerpta Medica International Congress*, Series No 66, 200–206.

Lee, C.N., Maurice, E., Ax, R.L., Pennington, J.A., Hoffman, W.F. and Brown, M.D. (1983) Efficacy of gonadotropin-releasing hormone administered at the time of artificial insemination of heifers and post-partum and repeat breeder dairy cows. *American Journal Veterinary Research*, **44**, 2160–2163.

Lee, L.R., Haisenleder, D.J., Marshall, J.C. and Smith, M.S. (1989a) Expression of alpha-subunit and luteinizing hormone (LH) beta messenger ribonucleic acid in the rat during lactation and after pup removal relationship to pituitary gonadotropin-releasing hormone receptors and pulsatile LH secretion. *Endocrinology*, **124**, 776–782.

Lee, L.R., Paul, S.J. and Smith, M.S. (1989c) Dose response effects of pulsatile GnRH administration on restoration of pituitary GnRH receptors and pulsatile LH secretion during lactation. *Neuroendocrinology*, **49**, 664–668.

Lee, L.R., Haisenleder, D.J., Marshall, J.C., Smith, M.S. (1989b) Effects of progesterone on pulsatile luteinizing hormone (LH) secretion and LH subunit messenger ribonucleic acid during lactation in the rat. *Endocrinology*, **124**, 2128–2134.

Legan, S.J., Coon, G.A., Karsch, F.J. (1975) Role of

estrogen as initiator of daily LH surges in the ovariectomized rat. *Endocrinology*, **96**, 50–56.

Le Maire, W.J., Shapiro, A.G., Riggall, F. and Yang, N.S.T. (1974) Temporary pituitary insensitivity to stimulation by synthetic LRF during the post-partum period. *Journal of Clinical Endocrinology and Metabolism*, **38**, 916–918.

Leung, K., Padmanabhan, V., Spicer, L.J., Tucker, H.A., Short, R.E. and Convey, E.M. (1986) Relationship between pituitary GnRH-binding sites and pituitary release of gonadotrophins in post-partum beef cows. *Journal of Reproduction and Fertility*, **76**, 53–63.

Lewis, G.S. and Bolt, D.J. (1987) Effects of suckling, progestogen-impregnated pessaries or hysterectomy on ovarian function in autumn-lambing post-partum ewes. *Journal of Animal Science*, **64**, 216–225.

Lewis, P.R., Brown, J.B. and Renfree, M.B. (1985) The resumption of ovulation and menstruation in a well-nourished population of women breastfeeding for an extended period of time. *Fertility and Sterility*, **55**, 529.

Lincoln, D.W. (1983) Physiological mechanisms governing the transfer of milk from mother to young, in *Symbiosis in Parent–Offspring Interactions*, (eds L.A. Rosenblum and H. Moltz), Plenum Publishing Corporation, New York pp. 77–112.

Lincoln, D.W. and Renfree, M.B. (1981a) Mammary gland growth and milk ejection in the agile wallaby, Macropus agilis, displaying concurrent asynchronous lactation. *Journal of Reproduction and Fertility*, **63**, 193–203.

Lincoln, D.W. and Renfree, M.B. (1981b) Milk ejection in a marsupial, Macropus agilis. *Nature*, **289**, 504–506.

Lincoln, D.W., Hill, A. and Wakerley, J.B. (1973) The milk-ejection reflex of the rat: an intermittent function not abolished by surgical levels of anaesthesia. *Journal of Endocrinology*, **57**, 459–476.

Lincoln, D.W., Hentzen, K., Hin, T., van der Schoot, P., Clarke, G. and Summerlee, A.J.S. (1980) Sleep: a pre-requisite for reflex milk ejection in the rat. *Experimental Brain Research*, **38**, 151–162.

Lincoln, D.W., Boyd, I.L., Pedersen, P., Kovaks, K. and Marwood, J. (1983) *Proceedings of 29th Congress of Physiological Sciences*, Sydney. Abstract 136.03.

Lincoln, D.W., Fraser, H.M., Lincoln, G.A., Martin, G.B. and McNeilly, A.S. (1985) Hypothalamic pulse generators. *Recent Progress in Hormone Research*, **41**, 369–411.

Lindblom, C., Sodersten, P. and Eneroth, P. (1985) Effects of pup sucking behaviour on inhibition of sexual behaviour and ovulatory secretion of LH in lactating rats. *Journal of Endocrinology*, **104**, 419–425.

Lishman, A.W., Allison, S.M.J., Fogwell, R.L., Butcher, R.L. and Inskeep, E.K. (1979) Follicular development and function of induced corpora lutea in underfed post-partum anestrous beef cows. *Journal of Animal Science*, **48**, 867–875.

Lodico, G., Stopelli, I., Delitala, G. and Maioli, M. (1983) Effects of naloxone infusion on basal and breast-stimulated prolactin secretion in puerperal women. *Fertility and Sterility*, **40**, 600–603.

Lomax, M.A., Baird, G.D., Mallinson, C.B. and Symonds, H.W. (1979) Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. *Journal of Biochemistry*, **180**, 281–289.

Loudon, A.I., McNeilly, A.S. and Milne, J.A. (1983) Nutrition and lactational control of fertility in red deer. *Nature*, **302**, 145–147.

Louw, B.P., Lishman, A.W., Botha, W.A., Arongie, P.A.R., Pultney, B.G. and Gunter, M.J. (1976) The release of luteinizing hormone (LH) in ewes deprived of prolactin during lactation. *South African Journal of Animal Science*, **6**, 87–91.

Lu, K.H., Chen, H.T., Grandison, L., Huang, H.H. and Meites, J. (1976a) Reduced luteinizing hormone release by synthetic luteinizing hormone-releasing hormone (LHRH) in post-partum lactating rats. *Endocrinology*, **98**, 1235–1240.

Lu, K.H., Chen, H.T., Huang, H.H., Grandison, L., Marshall, S. and Meites, J. (1976b) Relation between prolactin and gonadotropin secretion in post-partum lactating rats. *Journal of Endocrinology*, **68**, 241–250.

Lucas, A., Drewett, R.B. and Mitchell, M.D. (1980) Breastfeeding and plasma oxytocin concentrations. *British Medical Journal*, **281**, 834–835.

Lucy, M.C., Staples, C.R., Michel, F.M., Thatcher, W.W. and Bolt, D.J. (1991) Effect of feeding calcium soaps to early post-partum dairy cows on plasma prostaglandin F2 alpha, luteinizing hormone, and follicular growth. *Journal of Dairy Science*, **74**, 483–489.

Lunn, P.G. (1985) Maternal nutrition and lactational infertility: the baby in the driving seat, in *Maternal Nutrition and Lactational Infertility*, (ed.

J. Dobbing), Vevey/Raven Press, New York, pp. 41–53.

Lunn, P.G., Prentice, A.M., Austin, S. and Whitehead, R.G. (1980) Influence of maternal diet on plasma-prolactin levels during lactation. *Lancet*, **1**, 623–625.

Lunn, S.F. and McNeilly, A.S. (1982) Failure of lactation to have a consistent effect on interbirth interval in the common marmoset (*Callithrix jacchus jacchus*). *Folia Primatologica*, **37**, 99–105.

Lux-Lantos, V., Tesone, M. and Libertun, C. (1990) Ovarian follicle-stimulating hormone binding changes associated with the reinstatement of ovulatory cycles after lactation interruption in the rat. *Endocrinology*, **126**, 680–686.

McDowell, G.H., Hart, I.C. and Kirkby, A.C. (1987) Local intra-arterial infusion of growth hormone into the mammary glands of sheep and goats: effects on milk yield and composition, plasma hormones and metabolites. *Australian Journal of Biological Sciences*, **40**, 181–189.

McKinnon, Knight, C.H., Flint, D.J. and Wilde, C.J. (1988) Effect of milking frequency and efficiency on goat mammary prolactin receptor number. *Journal of Endocrinology*, **119**, Suppl 167.

McLeod, B.J., Haresign, W. and Lamming, G.E. (1982) Response of seasonally anoestrous ewes to small-dose multiple injections of Gn-RH with and without progesterone pretreatment. *Journal of Reproduction and Fertility*, **65**, 223–230.

McNatty, K.P., Sawers, R.S. and McNeilly, A.S. (1974) A possible role for prolactin in control of steroid secretion by the human graafian follicle. *Nature*, **250**, 653–655.

McNatty, K.P., Hudson, N., Gibb, M., Henderson, K.M., Lun, S., Heath, D. and Montgomery, G.W. (1984) Seasonal differences in ovarian activity in cows. *Journal of Endocrinology*, **102**, 189–198.

McNeilly, A.S. (1972) The blood levels of oxytocin during suckling and hand-milking in the goat with some observations on the pattern of hormone release. *Journal of Endocrinology*, **52**, 17–188.

McNeilly, A.S. (1977) Physiology of human lactation. *Journal of Biosocial Science*, Suppl. 4, 5–21.

McNeilly, A.S. (1979) Effects of lactation on fertility. *British Medical Bulletin*, **35**, 151–154.

McNeilly, A.S. (1980) Prolactin and the control of gonadotrophin secretion in the female. *Journal of Reproduction and Fertility*, **58**, 537–549.

McNeilly, A.S. (1984) Prolactin and ovarian function, in *Neuroendocrine Perspectives*, Vol. 3 (eds E.D. Muller and R.M. MacLeod), Elsevier Biomedical Press, Amsterdam, pp. 279–316.

McNeilly, A.S. (1985) Prolactin and the corpus luteum, in *The Luteal Phase*, (ed. S.L. Jeffcoate), John Wiley & Sons Ltd, Chichester, pp. 71–87.

McNeilly, A.S. (1987a) Suckling and the control of gonadotropin secretion, in *The Physiology of Reproduction*, (eds E. Knobil and J.D. Neill), Raven Press, New York.

McNeilly, A.S. (1987b) Prolactin and the control of gonadotrophin secretion. *Journal of Endocrinology*, **115**, 1–5.

McNeilly, A.S. (1993) Lactational Amenorrhoea. *Endocrinology and Metabolism Clinics of North America*, **22**, 59–73.

McNeilly, A.S. and Baird, D.T. (1983) Direct effect of prolactin induced by TRH injection, on ovarian oestradiol secretion in the ewe. *Journal of Reproduction and Fertility*, **69**, 559–568.

McNeilly, A.S. and Friesen, H.G. (1978) Prolactin levels during pregnancy and lactation in the rabbit. *Endocrinology*, **102**, 1548–1554.

McNeilly, A.S. and McNeilly, J.R. (1978) Spontaneous milk-ejection during lactation and its possible relevance to success of breastfeeding. *British Medical Journal*, **2**, 466–468.

McNeilly, A.S., Howie, P.W. and Houston, M.J. (1980) Relationships of feeding patterns, prolactin and resumption of ovulation post-partum, in *Research Frontiers in Fertility Regulation*, (eds G.I. Zatuchni, M.H. Labok and J.J. Sciarra), Harper & Row, New York, pp. 102–116.

McNeilly, A.S., Abbott, D.H., Lunn, S.F., Chambers, P.C. and Hearn, J.P. (1981) Plasma prolactin concentrations during the ovarian cycle and lactation and their relationship to return of fertility post-partum in the common marmoset (*Callithrix jacchus*). *Journal of Reproduction and Fertility*, **62**, 353–360.

McNeilly, A.S., Glasier, A., Jonassen, J. and Howie, P.W. (1982b) Evidence for direct inhibition of ovarian function by prolactin. *Journal of Reproduction and Fertility*, **65**, 559–569.

McNeilly, A.S., Howie, P.W., Houston, M.J., Cook, A. and Boyd, H. (1982a) Fertility after childbirth adequacy of post-partum luteal phases. *Clinical Endocrinology*, **17**, 609–615.

McNeilly, A.S., O'Connell, M. and Baird, D.T. (1982c) Induction of ovulation and normal luteal function by pulsed injections of luteinizing hormone in anestrous ewes. *Endocrinology*, **110**, 1292–1299.

McNeilly, A.S., Glasier, A.F., Howie, P.W., Houston, M.J., Cook, A. and Boyd, H. (1983c) Fertility after childbirth pregnancy associated with breast feeding. *Clinical Endocrinology*, **19**, 167–174.

McNeilly, A.S., Glasier, A., Swanston, I. and Djahanbakhch, O. (1983b) Prolactin and the human ovary, in *Human Prolactin Proceedings of the III International Congress*, (ed. G. Tolis), Raven Press, New York, pp. 173–178.

McNeilly, A.S., Martin, R.D., Hodges, J.K. and Smuts, G.L. (1983a) Blood concentrations of gonadotrophins, prolactin and gonadal steroids in male and in non-pregnant and pregnant female African elephants (*Loxodonta africana*). *Journal of Reproduction and Fertility*, **67**, 113–120.

McNeilly, A.S., Robinson, C.A.F., Houston, M.J. and Howie, P.W. (1983b) Release of oxytocin and prolactin in response to suckling. *British Medical Journal*, **286**, 257–259.

McNeilly, A.S., Glasier, A. and Howie, P.W. (1985) Endocrine control of lactational infertility, in *Maternal Nutrition and Lactational Infertility*, (ed. J. Dobbing), Vevey/Raven Press, New York, pp. 1–16.

McNeilly, J.R. (1972) Observations on the pattern of prolactin release during suckling in the ewe. *Journal of Reproduction and Fertility*, **31**, 487.

Madden, J.D., Boyar, R.M., MacDonald, P.C. and Porter, J.C. (1978) Analysis of secretory patterns of prolactin and gonadotropins during 24 hours in a lactating woman before and after resumption of menses. *American Journal of Obstetrics and Gynecology*, **132**, 436–441.

Madon, R.J., Ensor, D.M., Knight, C.H. and Flint, D.J. (1986a) Synergistic effects of growth hormone and prolactin on lactation, using a specific antiserum to rat growth hormone and bromocryptine. *Biochemical Society Transactions*, **14**, 330–331.

Madon, R.J., Ensor, D.M., Knight, C.H. and Flint, D.J. (1986b) Effects of an antiserum to rat growth hormone on lactation in the rat. *Journal of Endocrinology*, **111**, 117–123.

Madon, R.J., Ensor, D.M. and Flint, D.J. (1988) Effects of various secretagogues on insulin secretion *in vitro* of islets of Langerhans from virgin, pregnant and lactating rats. *Biochemical Society Transactions*, **13**, 878–879.

Maeda. K.I., Tsukamura, H., Uchida, E., Ohkura, N., Ohkura, S. and Yokoyama, A. (1989) Changes in the pulsatile secretion of LH after the removal of and subsequent resuckling by pups in ovariectomized lactating rats. *Journal of Endocrinology*, **121**, 277–283.

Maeda, K.I., Tsukamura, H., Ohkura, S., Kanaizuka, T. and Suzuki, J. (1991) Suppression of ovarian activity during the breeding season in suckling Japanese monkey (*Macaca fuscata*). *Journal of Reproduction and Fertility*, **92**, 371–375.

Mallinson, J.J.C. (1971) Observations on the reproduction and development of vervet monkey with special reference to intersubspecific hybridization (*cercopithecus pygerythrus* F. Cvier, 1921). *Mammalia*, **35**, 598–609.

Malven, P.V. and Hudgens, R.E. (1987) Naloxone-reversible inhibition of luteinizing hormone in post-partum ewes: effects of suckling and season. *Journal of Animal Science*, **65**, 196–202.

Maneckjee, R. and Moudgal, N.R. (1975) The onset of oestrus and ovulation in lactating rats. *Journal of Reproduction and Fertility*, **43**, 313–315.

Maneckjee, R., Srinath, B.R. and Moudgal, N.R. (1976) Prolactin suppresses release of luteinising hormone during lactation in the monkey. *Nature*, **262**, 507–508.

Markoff, E., Colosi, P. and Talamantes, F. (1981) Homologous radioimmunassay for secreted mouse prolactin. *Life Sciences*, **28**, 203–211.

Martal, J. (1975) Effets de la Fraite sur le Faux plasmatique de l'hormone somatotrope chez la brebis et chez la chevre. *Comptes Rendus Hebdomadaires des Séances de l'Academie de Sciences*, **D280**, 197–200.

Martin, M.J., Redmer, D.A., Ford, J.J., Christenson, R.K. and Day, B.N. (1986) Ovarian compensatory hypertrophy following unilateral ovariectomy in the suckled sow. *Journal of Animal Science*, **63**, 572–578.

Martin, R.D. (1966) Tree-shrews unique reproductive mechanism of systematic importance. *Science*, New York, **152**, 1402–1404.

Marynissen, G., Aerts, L. and van Assche, F.A. (1983) The endocrine pancreas during pregnancy and lactation in the rat. *Journal of Developmental Physiology*, **5**, 373–381.

Mattheij, J.A.M. and Swarts, H.J.M. (1987) Evidence that exteroceptive stimuli other than suckling have no major effect on plasma prolactin in lactating rats. *Acta Endocrinologica*, **114**, 417–425.

Mattioli, M. and Seren, E. (1985) Effect of bromocryptine treatment during lactational anestrus in pigs, in *Endocrine causes of seasonal and lactational anestrus in farm animals*, (eds F. Ellendorf, F.

Elsaesser), Martinus Nijhoff, The Netherlands, pp. 165–178.

Mattioli, M., Conte, F., Graleati, G. and Seren, E. (1986) Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. *Journal of Reproduction and Fertility*, **76**, 167–173.

Maule Walker, F.M. (1984) Exocrine and Endocrine Secretions of the Mammary Gland Local Control in Ruminants. *Symposium Zoological Society (London)*, **51**, 171–191.

Mayer, H., Schams, D., Worstoff, H. and Prokopp, A. (1984) Secretion of oxytocin and milk removal as affected by milking cows with and without manual stimulation. *Journal of Endocrinology*, **103**, 355–361.

Mayer, H., Schams, D., Worstorff, H. and Karg, H. (1986) Oxytocin release during prestimulation and milking and its significance for milk removal in dairy cows. *Vlaams Diergenees kunkig Tijdschrift*, **55**, 286–296.

Mena, F., Aguayo, D., Reeves, G. and Beyer, C. (1974) Effect of suckling and associated factors on ovarian compensatory hypertrophy during lactation in the rat. *Journal of Endocrinology*, **61**, 317–330.

Meyer, C., Freund-Mercier, Y., Guerne, Y. and Richard, P.L. (1987) Relationship between oxytocin release and amplitude of oxytocin cell neurosecretory bursts during suckling in the rat. *Journal of Endocrinology*, **114**, 263–270.

Minaguchi, H. and Meites, J. (1967) Effect of suckling on hypothalamic LH-releasing factor and prolactin inhibiting factor, and on pituitary LH and prolactin. *Endocrinology*, **80**, 603–607.

Mizoguchi, J. and Imamichi, T. (1986) Decrease in plasma insulin concentration in lactating rat. *Japanese Journal of Veterinary Science*, **48**, 837–839.

Mondain-Monval, M., Moller, O.M., Smith, A.J., McNeilly, A.S. and Scholler, R. (1985) Seasonal variations of plasma prolactin and LH concentrations in the female blue fox (*Alopex lagopus*). *Journal of Reproduction and Fertility*, **74**, 439–448.

Montgomery, G.W. (1982) Influence of suckling frequency and bromocryptine treatment on the resumption of ovarian cycles in post-partum beef cattle. *Theriogenology*, **17**, 551–563.

Montgomery, G.W., Davis, G.H. and Hurrell, G.A. (1980) Interval from calving to first oestrus in autumn- and spring-calving herds in the same locality. *Proceedings of the New Zealand Society of Animal Production*, **40**, 280–284.

Moody, N.W. and Speer, V.C. (1971) Factors affecting sow farrowing interval. *Journal of Animal Science*, **32**, 510–514.

Moore, K.E. (1987) Interactions between prolactin and dopaminergic neurons. *Biology of Reproduction*, **36**, 47–58.

Mori, M., Vigh, S., Miyata, A., Yoshihara, T., Oka, S. and Arimura, A. (1990) Oxytocin is the major prolactin releasing factor in the posterior pituitary. *Endocrinology*, **126**, 1009–1013.

Moss, G.E., Adams, T.E., Niswender, G.D. and Nett, T.M. (1980) Effects of pituitary and suckling on concentrations of pituitary gonadotropins, hypothalamic GnRH and pituitary responsiveness to GnRH in ewes. *Journal of Animal Science*, **50**, 496–502.

Moss, G.E., Parfet, J.R., Marvin, C.A., Allrich, R.D. and Diekman M.A. (1985) Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. *Journal of Animal Science*, **60**, 285–293.

Murai, C. and Ben-Jonathan, N. (1987) Posterior pituitary lobectomy abolishes the suckling-induced rist in prolactin (PRL): Evidence for a PRL-releasing factor in the posterior pituitary. *Endocrinology*, **121**, 205–211.

Murphy, L.J., Bell, G.I. and Friesen, H.G. (1987) Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. *Endocrinology*, **120**, 1279.

Murphy, M.G., Boland, M.P. and Roche, J.F. (1990) Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. *Journal of Reproduction and Fertility*, **90**, 523–533.

Myers, T.R., Myers, D.A., Gregg, D.W. and Moss, G.E. (1989) Endogenous opioid suppression of release of luteinizing hormone during suckling in post-partum anestrous beef cows. *Domesticated Animal Endocrinology*, **6**, 183–190.

Nagai, T., Makino, T., Ichida, N., Tanimoto, S. and Iizuka, R. (1986) The role of prolactin in lactating rats by passive immunization. *Asia-Oceania Journal of Obstetrics and Gynaecology*, **12**, 143–148.

Nagy, G., Kacsho, B. and Halasz, B. (1985) Episodic and pulsatile secretion of prolactin in the lactating rat. *Fidia Research*, Series 1, 123–130.

Nagy, G., Kacsoh, B., Kanyicska, B., Toth, B.E. and Korausz, E. (1986a) Separation and suckling-induced changes in serum growth

hormone levels in lactating rats and their pups. *Endocrinologica Experimentalis*, **20**, 217–222.

Nagy, G., Kacsoh, B. and Halasz, B. (1986b) Episodic prolactin and growth hormone secretion not related to actual suckling activity in lactating rats. *Journal of Endocrinology*, **111**, 137–142.

Nakano, R., Mori, A., Kayashima, F., Washio, M. and Tojo, S. (1975) Ovarian response to exogenously administered human gonadotropins during the post-partum period. *American Journal of Obstetrics and Gynecology*, **121**, 187–192.

Neill, J.D. (1986) Prolactin secretion and its control in *The Physiology of Reproduction*, (eds E. Knobil and J.D. Neill), Raven Press, New York, pp 1379–1390.

Newton, E.A., Stevenson, J.S., Minton, J.E. and Davis, D.L. (1987) Endocrine changes before and after weaning in response to boar exposure and altered suckling in sows. *Journal of Reproduction and Fertility*, **81**, 599–609.

Newton, M. (1961) Human Lactation in in *Milk: The Mammary Gland and its Secretions*, Vol. 1 (eds S.K. Kon and A.T. Cowie), Academic Press, New York. pp 281–320.

Noel, G.L., Suh, H.K. and Frantz, A.G. (1974) Prolactin release during nursing and breast stimulation in post-partum and non-post-partum subjects. *Journal of Clinical Endocrinology and Metabolism*, **38**, 413–423.

Nozaki, M., Watanabe, G., Taya, K., Katakai, Y., Wada, I. and Sasamoto, S. (1990) Changes in circulating inhibin levels during pregnancy and early lactation in the Japanese monkey. *Biology of Reproduction*, **43**, 444–449.

Nunley, W.C., Urban, R.J, Evans, W.S. and Veldhuis (1991) Preservation of pulsatile luteinizing hormone release during post-partum lactational amenorrhoea. *Journal of Clinical Endocrinology and Metabolism*, **73**, 629.

O'Byrne, K.T., Eltringham, L., Clarke, G. and Summerlee, A.J.S. (1986) Effects of porcine relaxin on oxytocin release from the neurohypophysis in the anaesthetized lactating rat. *Journal of Endocrinology*, **109**, 393–397.

Oxenreider, S.L. (1968) Effects of suckling and ovarian function on post-partum reproductive activity in beef cows. *American Journal of Veterinary Research*, **29**, 2099–2102.

Oxenreider, S.L. and Wagner, W.C. (1971) Effects of lactation and energy intake on al of post-partum ovarian activity in the cow. *Journal of Animal Science*, **33**, 1026–1031.

Paisley, A.C. and Summerlee, A.J.S. (1984) Activity of putative oxytocin neurones during relex milk ejection in conscious rabbits. *Journal of Physiology*, **347**, 465–478.

Palmer, W.M., Teague, H.S. and Venzke, W.G. (1965a) Histological changes in the reproductive tract of the sow during lactation and early post weaning. *Journal of Animal Science*, **24**, 1117–1125.

Palmer, W.M., Teague, H.S. and Venzke, W.G. (1965b) Microscopic observations on the reproductive tract of the sow during lactation and early post weaning. *Journal of Animal Science*, **24**, 541–545.

Parvizi, N., Elsaesser, F., Smidt, D. and Ellendorff, F. (1976) Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation and after ovariectomy and pentobarbitone treatment. *Journal of Endocrinology*, **69**, 193–203.

Payne, M.R., Howie, P.W., Cooper, W., Marnie, M., Kidd, L. and McNeilly, A.S. (1985) Sulpiride and the potentiation of progestogen only contraception. *British Medical Journal*, **291**, 559–561.

Peaker, M., Taylor, E., Tashima, L., Redman, T.L., Greenwood, F.C. and Bryant-Greenwood, G.D. (1989) Relaxin detected by immunocytochemistry and northern analysis in the mammary gland of the guinea pig. *Endocrinology*, **125**, 693.

Pelletier, J. and Thimonier, J. (1973) Comparison of the induced preovulatory LH discharge in lactating and dry sheep during seasonal anoestrus. *Journal of Reproduction and Fertility*, **33**, 310–313.

Perez, A., Vela, P., Masnick, G.S. and Potter, R.G. (1972) First ovulation after childbirth the effect of breast feeding. *American Journal of Obstetrics and Gynecology*, **114**, 1041–1047.

Perry, R.C., Corah, L.R., Cochran, R.C., Beal, W.E., Stevenson, J.S., Minton, J.E., Simms, D.D. and Brethour, J.R. (1991a) Influence of dietary energy on follicular development, serum gonadotropins, and first post-partum ovulation in suckled beef cows. *Journal of Animal Science*, **69**, 3762–3773.

Perry, R.C., Corah, L.R., Kiracofe, G.H., Stevenson, J.S., Beal, W.E. (1991b) Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of post-partum estrous cycles. *Journal of Animal Science*, **69**, 2548–2555.

Peters, A.R. (1984) Effect of exogenous oestradiol-

17β on gonadotrophin secretion in post-partum beef cows. *Journal of Reproduction and Fertility*, **72**, 473–478.

Peters, A.R. and Lamming, G.E. (1984) Reproductive activity of the cow in the post-partum period. II. Endocrine patterns and induction of ovulation. *British Veterinary Journal*, **140**, 269–280.

Peters, A.R. and Riley, G.M. (1982a) Milk progesterone profiles and factors affecting post-partum ovarian activity in beef cows. *Animal Production*, **34**, 145–153.

Peters, A.R. and Riley, G.M. (1982b) Is the cow a seasonal breeder? *British Veterinary Journal*, **138**, 533–537.

Peters, A.R., Lamming, G.E. and Fisher, M.W. (1981) A comparison of plasma LH concentrations in milked and suckling post-partum cows. *Journal of Reproduction and Fertility*, **62**, 567–573.

Peters, A.R., Pimentel, M.G. and Lamming, G.E. (1985) Hormone responses to exogenous GnRH pulses in post-partum dairy cows. *Journal of Reproduction and Fertility*, **75**, 557–565.

Peters, F., Delpozo, E., Conti, A. and Breckwoldt, M. (1986) Inhibition of lactation by a long-acting bromocriptine. *Obstetrics and Gynaecology*, **67**, 82–85.

Peters, R.R. and Tucker, H.A. (1978) Prolactin and growth hormone responses to photoperiod. *Endocrinology*, **103**, 229–234.

Pieper, D.R., Gala, R.R., Regiani, S.R. and Marshall, J.C. (1982) Dependence of pituitary gonadotropin-releasing hormone (GnRH) receptors on GnRH secretion from the hypothalamus. *Endocrinology*, **110**, 749–753.

Plant, K., Bauman, D.E., Agergaard, N. and Akers, R.M. (1987) Effect of exogenous prolactin administration on lactational performance of dairy cows. *Domestic Animal Endocrinology*, **4**, 279–290.

Plant, T.M., Schallenberger, E., Hess, D.L., McCormack, J.T., Dufy-Barbe, L. and Knobil, E. (1980) The influence of suckling on estrogen-induced gonadotropin discharges in the female rhesus monkey (*Macaca mulatta*). *Biology and Reproduction*, **23**, 760–766.

Plotsky, A.M. and Neill, J.D. (1982) The decrease in hypothalamic dopamine secretion induced by suckling: Comparison of voltammetric and radioisotopic methods of measurement. *Endocrinology*, **110**, 691–696.

Pohl, C.R., Lee, L.R. and Smith, M.S. (1989) Qualitative changes in luteinizing hormone and prolactin responses to N-methyl-aspartic acid during lactation in the rat. *Endocrinology*, **124**, 1905–1911.

Poindexter, A.N., Litter, M.B. and Besch, P.K. (1983) The recovery of normal plasma progesterone levels in the post-partum female. *Fertility and Sterility*, **39**, 494–498.

Porter, T.E., Ellerkmann, E. and Frawley, L.S. (1992) Estrogen-induced prolactin secretion. *Molecular and Cellular Endocrinology*, **84**, 23–31.

Postel-Vinay, M.C., Belair, L., Kayser, C., Kelly, P.A. and Djiane, J. (1991) Identification of prolactin and growth hormone binding proteins in rabbit milk. *Proceedings of the National Academy of Sciences of the USA*, **88**, 6687–6690.

Poulain, D.A., Rodriguez, F. and Ellendorf, F. (1981) Sleep is not a prerequisite for the milk ejection reflex in the pig. *Experimental Brain Research*, **43**, 107–110.

Prado, R., Rhind, S.M., Wright, I.A., Russell, A.J.F., McMillen, S.M., Smith, A.J. and McNeilly, A.S. (1990) Ovarian follicle populations, steroidogenicity and micromorphology at five and nine weeks post-partum in beef cows in two levels of body condition. *Animal Production*, **51**, 103–108.

Prosser, C.G., Fleet, I.R., Corps, A.N., Froesch, E.R. and Heap, R.B. (1990) Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. *Journal of Endocrinology*, **126**, 437–443.

Psychoyos, A. (1973) Hormonal control of ovoimplantation. *Vitamins and Hormones*, **31**, 201–256.

Quirk, S.J., Gannell, J.E., Fullerton, M.J. and Funder, J.W. (1986) Specificity and mechanism of biphasic action of glucocorticoids on α-lactalbumin production by rat mammary gland explants. *Endocrinology*, **118**, 909–914.

Quirk, S.J., Slattery, J. and Funder, J.W. (1990a) 11β-hydroxysteroid dehydrogenase activity in the mammary gland. *Journal of Steroid Biochemistry*, **35**, 623–625.

Quirk, S.J., Slattery, J.A. and Funder, J.W. (1990b) Epithelial and adipose cells isolated from mammary glands of pregnant and lactating rats differ in 11β-hydroxysteroid dehydrogenase activity. *Journal of Steroid Biochemistry*, **37**, 529–534.

Radford, H.M., Nancarrow, C.D. and Mattner, P.E. (1978) Evidence for hypothalamic dysfunction in suckled beef cows. *Theriogenology*, **6**, 641–646.

Rahe, C.M., Owens, R.E., Fleegar, J.L., Newton, H.J. and Harms, P.G. (1980) Pattern of plasma luteinising hormone in the cyclic cow: dependence upon the period of the cycle. *Endocrinology*, **107**, 498–503.

Ramirez, J.L., Bennett, W.A. and Cox, N.M. (1985) Effects of stage of lactation on estrous behaviour, LH and ovulation after administration of estradiol benzoate to sows (abstract). *Journal of Animal Science*, **61**, Suppl. 1, 44.

Ramirez-Godinez, J.A., Kiracofe, G.H., Schalles, R.R., Niswender, G.D. (1982) Endocrine patterns in the post-partum beef cow associated with weaning: a comparison of the short and subsequent normal cycles. *Journal of Animal Science*, **55**, 153–158.

Randel, R.D. (1990) Nutrition and post-partum rebreeding in cattle. *Journal of Animal Science*, **68**, 853–862.

Randel, R.D. and Welker, G.A. (1981) Effect of once-daily suckling on post-partum interval and cow-calf performance of first-calf Brahman X Hereford heifers. *Journal of Animal Science*, **53**, 755–757.

Randel, R.D., Short, R.E. and Bellows, R.A. (1976) Suckling effect on LH and progesterone in beef cows. *Journal of Animal Science*, **42**, 267–

Rawlings, N.C., Weir, L., Todd, B., Manns, J. and Hyland, J.H. (1980) Some endocrine changes associated with the post-partum period of the suckling beef cow. *Journal of Reproduction and Fertility*, **60**, 301–308.

Restall, B.J. and Starr, B.G. (1977) The influence of season on lambing and lactation on reproductive activity and plasma LH concentrations in Merino ewes. *Journal of Reproduction and Fertility*, **49**, 297–303.

Restall, B.J., Kearins, R.D. and Starr, B.G. (1977) Studies of pituitary function in lactating ewes. *Journal of Reproduction and Fertility*, **49**, 291–296.

Restall, B.J., Kearins, R.D., Herdegen, J. and Carberry, P. (1978) The induction of reproductive activity in lactating ewes. *Australian Journal of Agricultural Research*, **29**, 181–189.

Reyes, F.I., Winter, J.S.D. and Faiman, C. (1972) Pituitary–ovarian interrelationships during the puerperium. *American Journal of Obstetrics and Gynecology*, **714**, 589–594.

Rhind, S.M., Chesworth, J.M. and Robinson, J.J. (1978) A seasonal difference in ovine peripheral plasma prolactin and progesterone concentrations in early pregnancy and the relationship between the two hormones. *Journal of Reproduction and Fertility*, **52**, 79–81.

Rhind, S.M., Robinson, J.J., Chesworth, J.M. and Crofts, R.M.J. (1980) Effects of season, lactation and plane of nutrition on prolactin concentrations in ovine plasma and the role of prolactin in the control of ewe fertility. *Journal of Reproduction and Fertility*, **58**, 145–152.

Richardson, D.W., Goldsmith, L.T., Pohl, C.R., Schallenberger, E. (1985) The role of prolactin in the regulation of the primate corpus luteum. *Journal of Clinical Endocrinology and Metabolism*, **60**, 501–504.

Riis, P.M. and Madsen, A. (1985) Thyroxine concentrations and secretion rates in relation to pregnancy, lactation and energy balance in goats. *Journal of Endocrinology*, **107**, 421–427.

Riley, G.M., Peters, A.R. and Lamming, G.E. (1981) Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of GnRH. *Journal of Reproduction and Fertility*, **63**, 559–565.

Rimoin, D.L., Holzman, G.B., Merimee, T.J., Rabinowitz, D., Barnes, A.C., Tyson, J.E.A. and McKusick, V.A. (1968) Lacation in the absence of human growth hormone. *Journal of Clinical Endocrinology and Metabolism*, **28**, 1183–1188.

Rivero, R., Barrera, M. and Kennedy, K.I. (1988) Breastfeeding and the return to ovulation in Durango, Merico. *Fertility and Sterility*, **49**, 780.

Rivero, R., Ortiz, E., Barrera, M., Kennedy, K. and Bhiwandiwala, P. (1985) Preliminary observations on the return of ovarian function among breast-feeding and post-partum non-breast feeding women in a rural area of Mexico. *Journal of Biosocial Science*, Suppl. 9, 127–136.

Robinson, A.M., Girard, J.R. and Williamson, D.H. (1978) Evidence for a role of insulin in the regulation of lipogenesis in lactating rat mammary gland. Measurements of lipogenesis *in vivo* and plasma hormone concentrations in response to starvation and refeeding. *Biochemical Journal*, **176**, 343–348.

Robinson, I.C.A.F. and Jones, P.M. (1982) Oxytocin and neurophysin in plasma and cerebrospinal fluid during suckling in the guinea pig. *Neuroendocrinology*, **34**, 59–63.

Robinson, I.C.A.F, Woolf, C.N. and Parsons, J.A. (1981) Suckling in the guinea-pig: the simultaneous release of oxytocin and neurophysin. *Journal of Endocrinology*, **90**, 227–236.

Rojansthien, S.A.M., Lundeheim, N. and Einarsson, S. (1987) Luteinizing hormone re-

sponse to different doses of synthetic gonadotropin releasing hormone during early and late lactation in primiparous sows. *Animal Reproduction Science*, **13**, 299–307.

Rojkittikhun, T., Einarsson, S. and Kindahl, H. (1991) A technique for continuously monitoring hormone levels in lactating sows and results obtained using it to study LH release. *Zentralblatt für Veterinarmedizin Reihe A*, **38**, 344–349.

Rolland, R., Lequin, R.M., Schellekens, L.A. and De Jong, F.H. (1975) The role of prolactin in restoration of ovarian function during the early post-partum period in the human female. I. A study during physiological lactation. *Clinical Endocrinology*, **4**, 15–25.

Rothchild, I. (1960) The corpus luteum–pituitary relationship: the association between the cause of luteotrophin secretion and the cause of follicular quiescence during lactation: the basis for a tentative theory of the corpus luteum–pituitary relationship in the rat. *Endocrinology*, **67**, 9–41.

Rothchild, I. (1981) The regulation of the mammalian corpus luteum. *Recent Progress in Hormone Research*, **37**, 183–298.

Rowell, T.E. (1974) Female reproduction cycles and social behaviour in primates. *Advances in the Study of Behaviour*, **4**, 69–105.

Rund, L.A., Leshin, L.S., Thompson, F.N., Rampacek, G.B. and Kiser, T.E. (1989) Influence of the ovary and suckling on luteinizing hormone response to naloxone in post-partum beef cows. *Journal of Animal Science*, **67**, 1527–1533.

Rutter, L.M. and Manns, J.G. (1987) Hypoglycemia alters pulsatile luteinizing hormone secretion in the post-partum beef cow. *Journal of Animal Science*, **64**, 479–488.

Sagi, R., Gorewit, R.C., Merrill, W.G. and Wilson, D.B. (1980) Premilking stimulation effects on milking performance and oxytocin and prolactin release in cows. *Journal of Dairy Science*, **63**, 800–806.

Saiduddin, S., Riesen, W., Tyler, W. and Casida, L.E. (1968) Studies on the post-partum cow. 5.0.0. Relation of the post-partum interval to pituitary gonadotropins, ovarian follicular development and fertility in dairy cows. *Wisconsin Agricultural Experimental Station Bulletin*, **270**, 15.

Samson, W.K., Lumpkin, M.D. and McCann, S.M. (1986) Evidence for a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology*, **119**, 554–560.

Sar, M. and Meites, J. (1969) Effects of suckling on pituitary release of prolactin, GH, and TSH in post-partum rats. *Neuroendocrinology*, **4**, 25–31.

Savio, J.D., Boland, M.P., Hynes, N. and Roche, J.F. (1990a) Resumption of follicular activity in the early post-partum period of dairy cows. *Journal of Reproduction and Fertility*, **88**, 569–579.

Savio, J.D., Boland, M.P. and Roche, J.F. (1990b) Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. *Journal of Reproduction and Fertility*, **88**, 581–591.

Schallenberger, E. and Peterson, A.J. (1982) Effect of ovariectomy on tonic gonadotrophin secretion in cyclic and post-partum dairy cows. *Journal of Reproduction and Fertility*, **64**, 47–52.

Schallenberger, E., Schams, D. and Zottmeier, K. (1978) Response of lutropin (LH) and follitropin (FSH) to the administration of gonadolaberin (GnRH) in pregnant and post-partum cattle including experiments with prolactin suppression. *Theriogenology*, **10**, 35–53.

Schallenberger, E., Richardson, D.W. and Knobil, E. (1981) Role of prolactin in the lactational amenorrhoea of the Rhesus monkey (*Macaca mulatta*). *Biology and Reproduction*, **25**, 370–374.

Schallenberger, E., Oerterer, U. and Hutterer, G. (1982) Neuroendocrine regulation of post-partum function, in *Factors Influencing Fertility in the Post-partum Cow*, (eds H. Karg and E. Schallenberger), Martinuss Nijhoff, The Hague, pp. 123–147.

Schams, D., Hofer, F., Hoffman, B., Ender, M.L. and Karg, H. (1973) Effects of synthetic LH-RH treatment on bovine ovarian function during oestrous cycle and post-partum period. *Acta Endocrinologica*, Suppl. 177, 296.

Schams, D., Schallenberger, E., Menzer, C.H., Stangl, J., Zottmeser, K., Hoffman, B. and Karg, H. (1978) Profiles of LH, FSH and progesterone in post-partum dairy cows and their relationship to the commencement of cyclic function. *Theriogenology*, **10**, 453–468.

Schams, D., Mayer, H., Prokopp, A. and Horstorff, H. (1984) Oxytocin secretion during milking in dairy cows with regard to the variation and importance of a threshold level for milk removal. *Journal of Endocrinology*, **102**, 337–343.

Schemm, S.R., Deaver, D.R., Griel Jr., L.C. and Muller, L.D. (1990) Effects of recombinant bovine somatotropin on luteinizing hormone

and ovarian function in lactating dairy cows. *Biology of Reproduction*, **42**, 815–821.

Schirar, A., Cognie, Y., Louault, F., Poulin, N., Levasseur, M.C. and Martinet, J. (1989) Resumption of oestrous behaviour and cyclic ovarian activity in suckling and non-suckling ewes. *Journal of Reproduction and Fertility*, **87**, 789–94.

Schirar, A., Cognie, Y., Louault, F., Poulin, N., Meusnier, C., Levasseur, M.C. and Martinet, J. (1990) Resumption of gonadotrophin release during the post-partum period in suckling and non-suckling ewes. *Journal of Reproduction and Fertility*, **88**, 593–604.

van der Schoot, P. and Greef, W.J. de. (1986a) Development of ovarian follicles during lactation in rats. *Acta Endocrinologica*, **112**, 247–252.

van der Schoot, P. and Greef, W.J. de. (1986b) Prolactin and delay in implantation in lactating adrenalectomized rats. *Hormone Research*, **24**, 46–54.

van der Schoot, P., Lankhorst, R.R., de Roo, J.A. and de Greef, W.J. (1978) Suckling stimulus, lacatation and suppression of ovulation in the rat. *Endocrinology*, **103**, 949–956.

Schrick, F.N., Spitzer, J.C., Jenkins, T.C., Henricks, D.M. and Althen, T.G. (1990) Effect of dietary energy restriction on metabolic and endocrine responses during the estrous cycle of the suckled beef cow. *Journal of Animal Science*, **68**, 3313–3321.

Self, H.L. and Grummer, R.H. (1958) The rate and economy of pig gains and the reproductive behaviour in sows when litters are weaned at 10, 21 or 56 days. *Journal of Animal Science*, **17**, 862–868.

Selmanoff, M. and Gregerson, K.A. (1986) Suckling-induced prolactin release is suppressed by naloxone and stimulated by B endorphin. *Neuroendocrinology*, **42**, 255–259.

Selmanoff, M. and Wise, P.M. (1981) Decreased dopamine turnover in the median eminence in response to suckling in the lactating rat. *Brain Research*, **212**, 101–116.

Shaaban, M.M., Kennedy, K.I. and Sayed, G.H. (1990) The recovery of fertility during breastfeeding in Assuit, Egypt. *Journal of Biosocial Science*, **22**, 19.

Sharpe, P.H., McKibbin, P.E., Murphy, B.D. and Manns, J.G. (1986) First post-partum ovulations and corpora lutea in ewes which lamb in the breeding season. *Animal Reproduction Science*, **10**, 61–69.

Shaw, H.J. and Foxcroft, G.R. (1985) Relationships between LH, FSH and prolactin secretion and reproductive activity in the weaned sow. *Journal of Reproductive Fertility*, **75**, 17–28.

Sherwood, O.D., Chang, C.C., BeVier, G.D. and Dziak, P.G. (1975) Radioimmunoassay of plasma relaxin levels throughout pregnancy and at parturition in the pig. *Endocrinology*, **97**, 834–837.

Sherwood, O.D., Crnekovic, V.E., Gordon, W.L. and Rutherford, J.E. (1980) Radioimmunoassay of relaxin throughout pregnancy and during parturition in the rat. *Endocrinology*, **107**, 691–698.

Sherwood, O.D., Nara, B.S., Welk, F.A., First, N.L. and Rutherford, J.E. (1981) Relaxin levels in the maternal plasma of pigs before, during and after parturition and before, during and after suckling. *Biology of Reproduction*, **255**, 65–71.

Shevah, Y., Black, W.J.M. and Land, R.B. (1975) The effects of nutrition on the reproductive performance of Finn [40]X Dorset ewes. II. Post-partum ovarian activity, conception and the plasma concentration of progesterone and LH. *Journal of Reproduction*, **45**, 289–299.

Short, R.E., Bellows, R.A., Moody, E.L. and Howland, B.E. (1972) Effects of suckling and mastectomy on bovine post-partum reproduction. *Journal of Animal Science*, **34**, 70–74.

Short, R.E., Randel, R.D., Staigmiller, R.B. and Bellows, R.A. (1979) Factors affecting estrogen-induced LH release in the cow. *Biology and Reproduction*, **21**, 683–689.

Short, R.V. (1976) Lactation – the central control of reproduction, in *Breastfeeding and the Mother*, Ciba Foundation Symposium 45, Elsevier, Excerpta-Medica, Amsterdam, pp. 73–86.

Short, R.V., Lewis, P.R. and Renfree, M.B. (1991) Contraceptive effects of extended lactational amenorrhoea beyond the Bellagio consensus. *Lancet*, **337**, 715.

Sibaja, R.A. and Schmidt, G.H. (1975) Release of oxytocin in the cow during milking. *Journal of Dairy Science*, **58**, 569–570.

Simpson, M.J.A., Simpson, A.E., Hooley, J. and Zunz, M. (1981) Infant-related influences on birth intervals in rhesus monkey. *Nature*, **290**, 49–51.

Sinha, Y.N. (1992) *Prolactin variants. Trends in endocrinology and metabolism* **3**, 100–106.

Sinha, Y.N., Selby, F.W. and Vanderlaan, W.P. (1974) Relationship of prolactin and growth hor-

mone to mammary function during pregnancy and lactation in the C3H/ST mouse. *Journal of Endocrinology*, **61**, 219–229.

Sirinathsinghji, D.J.A. and Martini, L. (1984) Effects of bromocriptine and naloxone on plasma levels of prolactin, LH and FSH during suckling in the female rat responses to gonadotrophin releasing hormone. *Journal of Endocrinology*, **100**, 175–182.

Smith, D.M. (1961) The effect of daily separation of sows from their litters upon milk yield, creep intake and energetic efficiency. *New Zealand Journal of Agricultural Research*, **42**, 332–245.

Smith, J.F., Payne, E., Tervit, H.R., McGowan, L.T., Fairclough, R., Kilgour, R. and Goold, P.G. (1981) The effect of suckling upon the endocrine changes associated with anoestrus in identical twin dairy cows. *Journal of Reproduction and Fertility*, Suppl. 30, 241–249.

Smith, M.S. (1978a) A comparison of pituitary responsiveness to luteinizing hormone releasing hormone during lactation and the estrous cycle of the rat. *Endocrinology*, **102**, 114–120.

Smith, M.S. (1978b) The relative contribution of suckling and prolactin to the inhibition of gonadotropin secretion during lactation in the rat. *Biology of Reproduction*, **19**, 77–83.

Smith, M.S. (1981) Site of action of prolactin in the suppression of gonadotropin secretion during lactation in the rat: effect on pituitary responsiveness to LHRH. *Biology of Reproduction*, **24**, 967–976.

Smith, M.S. (1982a) Effect of pulsatile gonadotropin-releasing hormone on the release of luteinizing hormone and follicle-stimulating hormone *in vitro* by anterior pituitaries from lactating and cycling rats. *Endocrinology*, **110**, 882–891.

Smith, M.S. (1982b) Hypothalamic responsiveness during lactation in the rat: luteinizing hormone release after electrochemical stimulation. *Biology of Reproduction*, **27**, 125–131.

Smith, M.S. (1984) Effects of the intensity of the suckling stimulus and ovarian steroids on pituitary gonadotropin-releasing hormone receptors during lactation. *Biology of Reproduction*, **31**, 548–555.

Smith, M.S. (1985) Release of luteinizing hormone and follicle stimulating hormone from pituitaries of lactating rats by gonadotropin-releasing hormone and high potassium concentration. *Endocrinology*, **116**, 1826–1834.

Smith, M.S. and Lee, L.R. (1989) Modulation of pituitary gonadotropin-releasing hormone receptors during lactation in the rat. *Endocrinology*, **124**, 1456–1461.

Smith, M.S. and Neill, J.D. (1977) Inhibition of gonadotropin secretion during lactation in the rat: relative contribution of suckling and ovarian steroids. *Biology of Reproduction*, **17**, 255–261.

Smith, M.S., McClean, B.K. and Neill, J.D. (1976) Prolactin the initial luteotropic stimulus of pseudopregnancy in the rat. *Endocrinology*, **98**, 1370–1377.

Smith, V.G., Beck, T.W., Convey, E.M. and Tucker, H.A. (1974) Bovine serum prolactin, growth hormone, cortisol and milk yield after ergocryptine. *Neuroendocrinology*, **15**, 172–179.

Smith, V.G., Chenault, J.R., McAllister, J.F. and Lauderdale, J.W. (1987) Response of postpartum beef cows to exogenous progestogens and gonadotropin-releasing hormone. *Journal of Animal Science*, **64**, 540–551.

Sodersten, P. and Eneroth, P. (1984) Suckling and serum prolactin and LH concentrations in lactating rats. *Journal of Endocrinology*, **102**, 251–256.

Sodersten, P., Hansen, S. and Eneroth, P. (1983) Inhibition of sexual behaviour in lactating rats. *Journal of Endocrinology*, **99**, 189–197.

Sowa, M., Tsuji, K. and Nakano, R. (1986) Effects of sulpiride-induced hyperprolactinaemia on human ovarian follicles during the late follicular phase. *Fertility and Sterility*, **46**, 1032–1036.

Spicer, L.J., Leung, K., Convey, E.M., Gunther, J., Short, R.E. and Tucker, J.A. (1986a) Anovulation in post-partum suckled beef cows. I. Associations among size and numbers of ovarian follicles, uterine inovulation and hormones in serum and follicular fluid. *Journal of Animal Science*, **62**, 734–741.

Spicer, L.J., Convey, E.M., Tucker, J.A. and Echternkamp, S.E. (1986b) Effects of intermittent injections of LHRH on secretory patterns of LH and FSH and ovarian follicular growth during post-partum anovulation in suckled beef cows. *Journal of Animal Science*, **62**, 1317–1323.

Spicer, L.J., Convey, E.M., Leung, K., Short, R.E. and Tucker, H.A. (1986c) Anovulation in postpartum suckled beef cows. II. Associations among binding of 125I-labelled gonadotropins to granulosa and thecal cells, the concentration of steroids in serum and various sized ovarian follicles. *Journal of Animal Science*, **62**, 742–.

Spicer, L.J., Convey, E.M., Tucker, H.A. and Echternkamp, S.E. (1986d) Effects of inter-

mittent injections of LHRH on specific binding of 125I–labelled gonadotropins to granulosa and theca, and concentrations of steroids in serum and ovarian follicles during post-partum anovulation in suckled beef cows. *Journal of Animal Science*, **62**, 1324–1331.

Spicer, L.J. and Tucker, W.B. and Adams, G.D. (1990) Insulin-like growth factor-I in dairy cows relationships among energy balance, body condition, ovarian activity, and estrous behavior. *Journal of Dairy Science*, **73**, 929–937.

Steger, R.W., Matt, K.S., Klemcke, H.G. and Bartke, A. (1985) Interactions of photoperiod and ectopic pituitary grafts on hypothalamic and pituitary function in male hamsters. *Neuroendocrinology*, **41**, 89–96.

Steger, R.W. and Peluso, J.J. (1978) Gonadotrophin regulation in the lactating rat. *Acta Endocrinologica*, **88**, 668–675.

Steinetz, B.G., Goldsmith, L.T. and Lust, G. (1987) Plasma relaxin levels in pregnant and lactating dogs. *Biology of Reproduction*, **37**, 719–725.

Steinmetz, R.W., Grant, A.L. and Malven, P.V. (1993) Transcription of prolactin gene in milk secretory cells of the rat mammary gland. *Journal of Endocrinology*, **136**, 271–276.

Stern, J.M., Goldman, L. and Levine, S. (1973) Pituitary–adrenal responsiveness during lactation in rats. *Neuroendocrinology*, **12**, 179–191.

Stern, J.M., Konner, M., Herman, T.N. and Reichlin, S. (1986) Nursing behaviour, prolactin and post-partum amenorrheoa during prolonged lactation in American and Kung mothers. *Clinical Endocrinology*, **25**, 247–258.

Stevenson, J.S. and Britt, J.H. (1979) Relationships among luteinizing hormone estradiol, progesterone, glucocorticoids, milk yield, body weight and post-partum ovarian activity in holstein cows. *Journal of Animal Science*, **48**, 570–577.

Stevenson, J.S. and Britt, J.H. (1980) Luteinizing hormone, total estrogens and progesterone secretion during lactation and after weaning in sows. *Theriogenology*, **14**, 453–462.

Stevenson, J.S. and Britt, J.H. (1981) Interval to estrus in sows and the performance of pigs after alteration of litter size during late lactation. *Journal of Animal Science*, **53**, 177–181.

Stevenson, J.S. and Davis, D.L. (1984) Influence of reduced litter size and daily litter separation on fertility of sows at 2 to 5 weeks post-partum. *Journal of Animal Science*, **59**, 284–293.

Stevenson, J.S., Cox, N.M. and Britt, J.H. (1981) Role of the ovary in controlling luteinizing hormone, follicle stimulating hormone and prolactin secretion during and after lactation in pigs. *Biology of Reproduction*, **24**, 341–353.

Stewart, D.R. and Stabenfeldt, G. (1981) Relaxin activity in the pregnant mare. *Biology of Reproduction*, **25**, 281–289.

Stewart, D.R. and Stabenfeldt, G. (1985) Relaxin activity in the pregnant cat. *Biology of Reproduction*, **32**, 848–854.

Stewart, F. (1984) Mammogenesis and changing prolactin receptor concentrations in the mammary glands of the tammar wallaby (*Macropus eugenii*). *Journal of Reproduction and Fertility*, **71**, 141–148.

Summerlee, A.J.S. and Lincoln, D.W. (1981) Electrophysiological recordings from oxytocinergic neurones during suckling in the unanaesthetized lactating rat. *Journal of Endocrinology*, **90**, 255–265.

Summerlee, A.J.S., O'Byrne, K.T., Paisley, A.C., Breeze, M.F. and Porter, D.C. (1984) Relaxin affects the central control of oxytocin release. *Nature*, **309**, 372–374.

Summerlee, A.J.S., Paisley, A.C., O'Byrne, K.T., Fairhall, K.M., Robinson, I.C.A.F. and Fletcher, J. (1986) Aspects of the neuronal and endocrine components of reflex milk ejection in conscious rabbits. *Journal of Endocrinology*, **108**, 143–149.

Sutherland, R.C., Aizlewood, E.S. and Wakerley, J.B. (1986) Changing characteristics of the milk-ejection reflex during pregnancy, lactation and after weaning in the rat. *Journal of Reproduction and Fertility*, **76**, 123–130.

Sutter-Dub, M.T., Leclerq, R., Sutter, B.C. and Jacquot, R. (1974) Plasma glucose, progesterone and immunoreactive insulin levels in the lactating rat. *Hormone and Metabolic Research*, **6**, 297–302.

Sutton, J.D., Hart, I.C., Broster, W.H., Elliott, R. and Schuller, E. (1986) Feeding frequency for lactating cows: effects on rumen fermentation and blood metabolites and hormones. *British Journal of Nutrition*, **56**, 181–192.

Suzuki, O. and Sato, M. (1984) Effects of once-daily suckling and twice-daily suckling on post-partum reproductive performance of beef cows and on growth and ingestive behaviour of their calves. *Japan Journal of Animal Production*, **30**, 39–45.

Taverne, M., Bevers, M., Bradshaw, J.M.C., Dieleman, S.J., Willamse, A.H. and Porter,

D.G. (1982) Plasma concentrations of prolactin, progesterone, relaxin and oestradiol-17β in sows treated with progesterone, bromocriptine or indomethacin during late pregnancy. *Journal of Reproduction and Fertility*, **65**, 85–96.

Tay, C.C.K., Glasier, A.F. and McNeilly, A.S. (1992) The twenty-four hour pattern of pulsatile luteinizing hormone, follicle stimulating hormone and prolactin release during the first eight weeks of lactational amennorhoea in breastfeeding women. *Human Reproduction*, **7**, 951–958.

Tay, C.C.K., Glasier, A.F. and McNeilly, A.S. (1993) Effect of antagonists of dopamine and opiates on the basal and GnRH-induced secretion of luteinizing hormone, follicle stimulating hormone and prolactin during lactational amenorrhoea in breastfeeding women. *Human Reproduction*, **8**, 532–539.

Taya, K. and Greenwald, G.S. (1982) Mechanisms of suppression of ovarian follicular development during lactation in the rat. *Biology of Reproduction*, **27**, 1090–1101.

Taya, K. and Sasamoto, S. (1980) Initiation of follicular maturation and ovulation after removal of the litter from the lactating rat. *Journal of Endocrinology*, **87**, 393–400.

Taya, K. and Sasamoto, S. (1987) Difference in the response of follicle maturation and ovulation between early and late lactating rats after removal of the litter. *Journal of Endocrinology*, **113**, 271–276.

Taya, K. and Sasamoto, S. (1988a) Induced development of ovulatory follicles during the early stages of lactation by the administration of LH in the rat. *Journal of Endocrinology*, **116**, 115–122.

Taya, K. and Sasamoto, S. (1988b) Selective release of FSH in lactating rats during the period of follicular atresia induced by the administration of antiserum to LH-releasing hormone. *Journal of Endocrinology*, **118**, 455–464.

Taya, K. and Sasamoto, S. (1989) Inhibitory effects of corticotrophin-releasing factor and beta-endorphin on LH and FSH secretion in the lactating rat. *Journal of Endocrinology*, **120**, 509–515.

Taya, K. and Sasamoto, S. (1990) Involvement of the adrenal gland in the suckling-induced decrease in LH and FSH secretion and the concomitant increase in prolactin secretion in the rat. *Journal of Endocrinology*, **125**, 279–285.

Taya, K. and Sasamoto, S. (1991) Mechanisms responsible for suppression of FSH and LH during lactation in the rat. *Journal of Endocrinology*, **129**, 119–30.

Taya, K., Komura, H., Watanabe, G. and Sasamoto, S. (1989) Peripheral blood levels of immunoreactive inhibin during pseudopregnancy, pregnancy and lactation in the rat (published erratum appears in *Journal of Endocrinology* **122**, 770). *Journal of Endocrinolology*, **121**, 545–552.

Taylor, J.C. and Peaker, M. (1975) Effects of bromocriptine on milk secretion in the rabbit. *Journal of Endocrinology*, **67**, 313–314.

Terkel, J., Blake, C.A. and Sawyer, C.H. (1972) Serum prolactin levels in lactating rats after suckling or exposure to ether. *Endocrinology*, **91**, 49–53.

Thapa, S., Short, R.V. and Potts, M. (1988) Breast feeding, birth spacing and their effects on child survival. *Nature*, **335**, 679.

Thomas, G.B., Cummins, J.T., Canny, B.J., Rundle, S.E., Griffin, N., Katsahambas, S. and Clarke, I.J. (1989) The posterior pituitary regulates prolactin, but not adrenocorticotropin or gonadotropin, secretion in the sheep. *Endocrinology*, **125**, 2204–2211.

Thompson, F.N., Caudle, A.B., Kemppainen, R.J., Nett, T.M., Brown, J. and Williams, D.J. (1986) Thyroidal and prolactin secretion in agalactic mares. *Theriogenology*, **25**, 575–580.

Thompson, L.H., Hanford, K.J. and Jensen, A.H. (1981) Estrus and fertility in lactating sows and piglet performance as influenced by limited nursing. *Journal of Animal Science*, **53**, 1419–1423.

Thordarson, G., Holekamp, K.E. and Talamantes, F. (1987) Development of a homologous radioimmunoassay for secreted prolactin from the california ground squirrel (*Spermophilus beecheyi*). *Biology of Reproduction*, **36**, 1186–1190.

Tindal, J.S. and Yokoyama, A. (1962) Assay of oxytocin by the milk ejection response on the anaesthetised lactating guinea pig. *Endocrinology*, **71**, 196–202.

Tomogane, H., Ota, K. and Yokoyama, A. (1969) Progesterone and 20 -hydroxypregn-4-en-3-one levels in ovarian vein blood of the rat throughout lactation. *Journal of Endocrinology*, **44**, 101–106.

Travis, J.C. and Holmes, W.N. (1974) Some physiological and behavioural changes associated with oestrus and pregnancy in the squirrel monkey (*Saimiri scuireus*). *Journal of Zoology*, (London), **174**, 41–66.

Tribble, R.L., Sorensen, A.M., Woodward, T.L., Connor, J.S., Beverly, J.R. and Fleeger, J.L. (1973) Serum progestins and luteinizing hor-

mone levels in non-suckled primiparous heifers. *Nature*, (London), **246**, 494–495.

Troxel, T.R., Kesler, D.J., Noble, R.C. and Carlin, S.E. (1980) Ovulation and reproductive hormones following steroid pretreatment, calf removal and GnRH in post-partum suckled beef cows. *Journal of Animal Science*, **51**, 652–659.

Tsukamura, H., Maeda, K.I. and Yokoyama, A. (1988) Effect of the suckling stimulus on daily LH surges induced by chronic oestrogen treatment in ovariectomized lactating rats. *Journal of Endocrinology*, **118**. 311–316.

Tsushima, T., Irie, M. and Sakuma, K. (1971) Radioimmunoassay for canine growth hormone. *Endocrinology*, **89**, 685–693.

Tucker, H.A. and Merkel, R.A. (1987) Applications of hormones in the metabolic regulation of growth and lactation in ruminants. *Federation Proceedings*, **46**, 300–306.

Tutin, C.E.G. (1980) Reproductive behaviour of wild chimpanzees in the Gombe National Park, Tanzania. *Journal of Reproduction and Fertility*, **28**, 43–57.

Tyndale-Biscoe, C.H. (1984) Mammals marsupials, in *Marshall's Physiology of Reproduction*, 4th edn, Vol 1, Chapter 6 (ed. G.E. Lamming), Churchill Livingstone, Edinburgh, pp. 386–454.

Tyndale-Biscoe, C.H. and Hawkins, J. (1977) The corpora lutea of marsupials aspects of function and control, in *Reproduction and Evolution Australian Academy of Science*, (eds J.H. Calaby and C.H. Tyndale-Biscoe), Canberra, pp. 245–252.

Tyndale-Biscoe, C.H. and Hinds, L.A. (1984) Seasonal patterns of circulating progesterone and prolactin in response to bromocriptine in the female tammar (*Macropus eugenii*). *General and Comparative Endocrinology*, **53**, 58–68.

Tyndale-Biscoe, C.H., Hinds, L.A. and McConnell, S.J. (1986) Seasonal breeding in a marsupial opportunities of a new species for an old problem. *Recent Progress in Hormone Research*, **42**, 471–507.

Tyson, J.E., Friesen, H.G. and Anderson, M.S. (1972) Human lactational and ovarian response to endogenous prolactin release. *Science*, **177**, 897–900.

Tyson, J.E., Carter, J.N., Andreassen, B., Huth, J. and Smith, B. (1978) Nursing-mediated prolactin and luteinizing hormone secretion during puerperal lactation. *Fertility and Sterility*, **30**, 154–162.

Udesky, I.C. (1950) Ovulation in lactating women. *American Journal of Obstetrics and Gynecology*, **59**, 843–848.

Uilenbroek, J.Th.J., van der Schoot, P., Den Besten, D. and Lankhorst, R.R. (1982) A possible direct effect of prolactin on follicular activity. *Biology of Reproduction*, **27**, 1119–1125.

Unvas-Moberg, K., Widstrom, A.M., Werner, S., Mattiesen, A.S and Winberg, J. (1990) Oxytocin and prolactin levels in breast-feeding women. Correlation with milk yield and duration of breast-feeding. *Acta Obstetrica and Gynecologica Scandanavica*, **69**, 301.

Vandenberg, J.G. and Vessey, S. (1968) Seasonal breeding of free-ranging rhesus monkeys and related ecological factors. *Journal of Reproductive Fertility*, **15**, 71–79.

Varley, M.A. and Vessey, S.H. (1977) Effects of geographic transfer on the timing of seasonal breeding of rhesus monkeys. *Folio Primatology*, **28**, 52–59.

Vermer, H.M. and Rolland, R. (1982) The influence of exogenous oestradiol benzoate on the pituitary responsiveness to LHRH during the puerperium in women. *Clinical Endocrinology*, **16**, 251–258.

Vernon, R.G. (1989) Endocrine control of metabolic adaptation during lactation. *Proceedings of the Nutritional Society*, **48**, 23–32.

Vernon, R.G. (1991) Depot specific endocrine control of fatty acid synthesis in adipose tissues of foetal lambs. *Domestic Animal Endocrinology*, **8**, 161–164.

Vernon, R.G., Finley, E. and Watt, P.W. (1991) Adenosine and the control of adrenergic regulation of adipose tissue lipolysis during lactation. *Journal of Dairy Science*, **74**, 695–705.

Voloschin, L.M. and Tramezzani, J.H. (1984) Relationship of prolactin release in lactating rats to milk ejection, sheep state, and ultrasonic vocalization by the pups. *Endocrinology*, **114**, 618–623.

Voogt, J.L., Sar, M. and Meites, J. (1969) Influence of cycling, pregnancy, labor and suckling on corticosterone-ACTH levels. *American Journal of Physiology*, **216**, 655–658.

Vonderhaar, B.K. and Ziska, S.E. (1989) Hormonal regulation of milk protein gene expression. *American Review of Physiology*, **1**, 641–652.

Vostermans, J.P.M. and Walton, J.S. (1985) Effect of intermittent injections of gonadotrophin releasing hormone at various frequencies on the release of luteinizing hormone and ovulation in

dairy cows. *Animal Reproductive Science*, **8**, 335–347.

Wakerley, J.B., Dyball, R.E.J. and Lincoln, D.W. (1973) Milk ejection in the rat: the result of selective release of oxytocin. *Journal of Endocrinology*, **57**, 557–558.

Walker, C. and England, D. (1978) Reproduction and litter performance of sows mated during lactation. *Western Section American Social Animal Science*, **29**, 115–116.

Walters, D.L., Kaltenback, C.C., Dunn, T.G. and Short, R.E. (1982) Pituitary and ovarian function in post-partum beef cows. 1. Effect of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. *Biology of Reproduction*, **26**, 640–646.

Walton, J.S., McNeilly, J.R., McNeilly, A.S. and Cunningham, F.J. (1977) Changes in blood levels of prolactin, LH, FSH and progesterone during anoestrus in the ewe. *Journal of Endocrinology*, **75**, 127–136.

Webb, R. and Lamming, G.E. (1981) Patterns of plasma prolactin in post-partum suckled cows. *Journal of Endocrinology*, **90**, 391–396.

Webb, R., Lamming, G.E., Haynes, N.B., Hafs, H.D. and Manns, J.G. (1977) Response of cyclic and post-partum suckled cows to injections of synthetic LH-RH. *Journal of Reproduction and Fertility*, **50**, 203–210.

Webb, R., Lamming, G.E., Hannes, N.B. and Foxcroft, G.R. (1980) Plasma progesterone and gonadotrophin concentrations and ovarian activity in post-partum dairy cows. *Journal of Reproduction and Fertility*, **59**, 133–143.

Weiss, G. (1984) Relaxin. *Annual Reviews of Physiology*, **46**, 43–52.

Weiss, G., Dierschke, D.J., Karsch, F.J., Hotchkiss, J., Butler, W.R. and Knobil, E. (1973) The influence of lactation on luteal function in the Rhesus monkey. *Endocrinology*, **93**, 954–959.

Weiss, G., Butler, W.R., Hotchkiss, J., Dierschke, D.J. and Knobil, E. (1976a) Periparturitional serum concentrations of prolactin, the gonadotrophins and the gonadal hormones in the Rhesus monkey. *Proceedings of the Society for Experimental Biology and Medicine*, **151**, 113–116.

Weiss, G., Butler, W.R., Dierschke, D.J. and Knobil, E. (1976b) Influence of suckling on gonadotropin secretion in the post-partum rhesus monkey. *Proceedings of the Society for Experimental Biology and Medicine*, **153**, 330–331.

Weist, J.J. and Thompson Jr., D.L. (1987) Plasma prolactin, LH and FSH concentrations after suckling in post-partum mares. *Journal of Reproduction and Fertility*, Suppl 35, 731–732.

Weitzman, R.E., Leake, R.D., Rubin, R.T. and Fisher, D.A. (1980) The effect of nursing on neurohypophyseal hormone and prolactin secretion in human subjects. *Journal of Clinical Endocrinology and Metabolism*, **51**, 836–839.

Wetterman, R.P., Turman, E.J., Wyatt, R.D. and Totusek, R. (1978) Influence of suckling intensity on reproductive performance of range cows. *Journal of Animal Science*, **47**, 342–346.

Wheeler, M.B., Anderson, G.B., Munro, C.J. and Stabenfeldt, G.H. (1982) Prolactin response in beef cows and heifers suckling one or two calves. *Journal of Reproduction and Fertility*, **64**, 243–249.

Whisnant, C.S., Kiser, T.E. and Thompson, F.N. (1985) Effect of calf removal on serum luteinizing hormone and cortisol concentrations in post-partum beef cows. *Theriogenology*, **24**, 119–129.

Whisnant, C.S., Thompson, F.N., Kiser, T.E. and Barb, C.R. (1986a) Effect of naloxone on serum luteinizing hormone, cortisol and prolactin concentrations in anestrous beef cows. *Journal of Animal Science*, **62**, 1340–1345.

Whisnant, C.S., Kiser, T.E., Thompson, F.N. and Barb, C.R. (1986b) Opioid inhibition of luteinizing hormone secretion during the post-partum period in suckled beef cows. *Journal of Animal Science*, **63**, 1445–1448.

Whisnant, C.S., Kiser, T.E., Thompson, F.N. and Barb, C.R. (1986c) Influence of calf removal on the serum luteinizing hormone response to naloxone in the post-partum beef cow. *Journal of Animal Science*, **63**, 561–564.

Whitely, J., Willcox, D.L., Hartmann, P.E., Yamamoto, S.Y., and Bryant-Greenwood, G.D. (1985) Plasma relaxin levels during suckling and oxytocin stimulation in the lactating sow. *Biology of Reproduction*, **33**, 705–714.

van de Wiel, D.F.M., van Landeghem, A.A.J., Willemse, A.H. and Bevers, M.M. (1979) Endocrine control of ovarian function after weaning in the domestic sow (abstract). *Journal of Endocrinology*, **80**, 69.

Williams, G.L. (1990) Suckling as a regulator of post-partum rebreeding in cattle: a review. *Journal of Animal Science*, **68**, 831–852.

Williams, G.L. and Ray, D.E. (1980) Hormonal and reproductive profiles of early post-partum beef heifers after prolactin suppression or steroid-induced luteal function. *Journal of Animal Science*, **50**, 906–918.

Williams, G.L., Kotwica, J., Slanger, W.D., Olson, D.K., Tilton, J.E. and Johnson, L.J. (1982) Effect of suckling on pituitary responsiveness to gonadotropin-releasing hormone throughout the early post-partum period of beef cows. *Journal of Animal Science*, **54**, 594–602.

Williams, G.L., Talavera, F., Petersen, B.J., Kirsch, J.D. and Tilton, J.E. (1983) Coincident secretion of follicle-stimulating hormone and luteinzing hormone in early post-partum beef cows: effect of suckling and low-level increases in systemic progesterone. *Biology of Reproduction*, **29**, 362–373.

Williams, G.L., Kirsch, J.D., Post, G.R., Tilton, J.E. and Slanger, W.D. (1984) Evidence against chronic teat stimulation as an autonomous effector of dimished gonadotropin release in beef cows. *Journal of Animal Science*, **59**, 1060–1069.

Williams, G.L., Koziorowski, M., Osborn, R.G., Kirsch, J.D. and Slanger, W.D. (1987) The post-tweaning rise of tonic luteinizing hormone secretion in anoestrous cows is not prevented by chronic milking or the physical presence of the calf. *Biology of Reproduction*, **36**, 1079–1084.

Williams, R.F. and Hodgen, G.D. (1983) Mechanism of lactational anovulation in primates: nursing increases ovarian steroidogenesis, in *Factors Regulating Ovarian Function*, (eds G.S. Greenwald and P.D. Terranova), Raven Press, New York, pp. 17–21.

Williams, R.F., Johnson, D.K. and Hodgen, G.D. (1979) Resumption of estrogen-induced gonadotropin surges in post-partum monkeys. *Journal of Clinical Endocrinology and Metabolism*, **49**, 422–428.

Wilson, M.E., Walker, M.L. and Pope, N.S. (1988) Prolonged lactational infertility in adolescent rhesus monkeys. *Biology of Reproduction*, **38**, 163–174.

Wiltbank, J.N. and Cook, A.C. (1958) The comparative reproductive performance of nursed cows and milked cows. *Journal of Animal Science*, **17**, 640–648.

Wiltbank, J.N., Rowden, W.W., Ingalls, J.E. and Zimmerman, D.R. (1964) Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. *Journal of Animal Science*, **23**, 1049–1053.

Wise, M.E. (1990) Gonadotropin-releasing hormone secretion during the post-partum anestrous period of the ewe. *Biology of Reproduction*, **43**, 19–725.

Wise, M.E., Nilson, J.E., Nejedlik, M.T. and Nett, T.M. (1985) Measurement of messenger RNA for luteinizing hormone β-subunit and α-subunit during gestation and the post-partum period in ewes. *Biology of Reproduction*, **33**, 1009–1015.

Wise, M.E., Glass, J.D. and Nett, T.M. (1986) Changes in the concentration of hypothalamic and hypophyseal receptors for estrodiol in pregnant and post-partum ewes. *Journal of Animal Science*, **62**, 1021–1028.

Wolf, R.H., Harnison, R.M. and Martin, T.W. (1975) A review of reproductive patterns in New World monkeys. *Laboratory Animal Science*, **25**, 814–821.

Wood, J.W., Johnson, P.L. and Campbell, K.L. (1985a) Demographic and endocrinological aspects of low natural fertility in highland New Guinea. *Journal of Biosocial Science*, **17**, 57–79.

Wood, J.W., Lai, D., Johnson, P.L., Campbell, K.L. and Maslar, I.A. (1985b) Lactation and birth spacing in highland New Guinea. *Journal of Biosocial Science*, Suppl. 9, 159–173.

Worthy, K., Escreet, R., Rentonm, J.P., Eckershall, P.D., Douglas, T.A. and Flint, D.J. (1986) Plasma prolactin concentrations and cyclic activity in pony mares during parturition and early lactation. *Journal of Reproduction and Fertility*, **77**, 569–574.

Wright, I.A., Rhind, S.M., Russel, A.J.F., Whyte, T.K., McBean, A.J. and McMillen, S.R. (1987) Effects of body condition, food intake and temporary calf separation on the duration of the post-partum anoestrus period and associated LH, FSH and prolactin concentrations in beef cows. *Animal Production*, **45**, 395–402.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J. and Findlay, J.K. (1980) Pituitary responsiveness to LH-RH, the occurrence of oestradiol -17β induced LH positive feedback and the resumption of oestrous cycles in ewes post-partum. *Journal of Reproduction and Fertility*, **60**, 171–176.

Wright, P.J., Findlay, J.K. and Anderson, G.A. (1981b) The failure of prolactin to enhance the inhibitory effect of oestradiol-17β on LH synthesis and release in ewes. *Journal of Reproduction and Fertility*, **62**, 537–542.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J. and Findlay, J.K. (1981b) Evidence for a change in oestradiol negative feedback and LH pulse frequency in post-partum ewes. *Journal of Reproduction and Fertility*, **61**, 97–102.

Wright, P.J., Jenkin, G. and Heap, R.B. (1981c)

Prolactin and LH release in response to LHRH and TRH in ewes during dioestrus, pregnancy and post-partum. *Journal of Reproduction and Fertility*, **62**, 447–453.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J. and Findlay, J.K. (1983) LH release and luteal function in post-partum acyclic ewes after the pulsatile administration of LH-RH. *Journal of Reproduction and Fertility*, **67**, 257–262.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J. and Findlay, J.K. (1984) Induction of plasma LH surges and normal luteal function in acyclic post-partum ewes by the pulsatile administration of LH-RH. *Journal of Reproduction and Fertility*, **71**, 1–6.

Wu, T.J., McArthur, N.H. and Harms, P.G. (1992) A possible dual mechanism involved in the inhibitory influence of suckling on luteinizing hormone release in the ovariectomized rat. *Biology of Reproduction*, **46**, 653–657.

Yamaji, T., Dierschke, D.J., Hotchkiss, J., Bhatacharya, A.N., Surve, A.H. and Knobil, E. (1971) Estogen induction of LH release in the rhesus monkey. *Endocrinology*, **89**, 1034–1041.

Ylikorkala, O., Kauppila, A., Kivinen, S. and Viinikka, L. (1982) Sulpiride improves inadequate lactation. *British Medical Journal*, **285**, 249–251.

Yohkaichiya, T., O'Connor, A. and de-Kretser, D.M. (1991) Circulating immunoreactive inhibin, gonadotropin, and prolactin levels during pregnancy, lactation, and postweaning estrous cycle in the rat. *Biology of Reproduction*, **44**, 6–12.

Yoshinaga, K. (1974) Ovarian progestin secretion in lactating rats effects of intrabursal injection of prolactin antiserum prolactin and LH. *Endocrinology*, **94**, 829–834.

Yoshinaga, K., Moudgal, N.R. and Greep, R.O. (1971) Progestin secretion by the ovary in lactating rats effect of LH-antiserum, LH and prolactin. *Endocrinology*, **88**, 1126–1135.

Zaied, A.A., Garverick, H.A., Bierschwal, C.J., Elmore, R.G., Youngquist, R.S. and Sharp, A.J. (1980) Effect of ovarian activity and endogenous reproductive hormones on GnRH-induced ovarian cycles in post-partum dairy cows. *Journal of Animal Science*, **50**, 508–513.

Zalesky, D.D., Forrest, D.W., McArthur, N.H., Wilson, J.M., Morris, D.L. and Harms, P.G. (1990) Suckling inhibits release of luteinizing hormone-releasing hormone from the bovine median eminence following ovariectomy. *Journal of Animal Science*, **68**, 444–448.

Zanartu, J., Dabancens, A., Kastin, A.J. and Schally, A.V. (1974) Effect of synthetic hypothalamic gonadotropin-releasing hormone (FSH/LII-RH) in anovulatory sterility. *Fertility and Sterility*, **25**, 160–169.

Zarate, A., Canales, E.S., Soria, J., Ruiz, F. and MacGregor, C. (1972) Ovarian refractoriness during lactation in women: effect of gonadotropin stimulation. *America Journal of Obstetrics and Gynecology*, **112**, 1130–1132.

Zarrow, M.X., Denenberg, V.H. & Anderson, C.O. (1965) Rabbit: frequency of suckling in the pup. *Science*, N.Y. **150**, 1835–1836

Zarrow, H.X., Schlein, P.A., Denenberg, V.H. and Cohen, H.A. (1972) Sustained corticosterone release in lactating rats following olfactory stimulation from the pups. *Endocrinology*, **91**, 191–

Ziegler, T.E., Widowski, T.M., Larson, M.L. and Snowdon, C.T. (1990) Nursing does affect the duration of the post-partum to ovulation interval in cotton-top tamarins *(Saguinus oedipus)*. *Journal of Reproduction and Fertility*, **90**, 563–570

PHYSIOLOGY AND BIOCHEMISTRY OF LACTATION

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12.1 INTRODUCTION

Cohen and Massey (1984) have pointed out that in animal reproduction 'much more passes between the generations than simple information coded for by DNA'. In mammals, quite apart from factors such as 'cultural inheritance', the observation acquires tangible validity in the secretion of milk. It is sometimes overlooked that lactation is part of reproduction. Perhaps because, for over a century, increasing numbers of women have abandoned breastfeeding in favour of bottle feeding, lactation has come to be seen as an 'optional extra'. There are very serious grounds for questioning that perception, particularly under the social and environmental conditions in which the vast majority of human babies are reared. Indeed, artificial feeding is most appropriately compared with certain other technological innovations, like artificial insemination – invaluable on occasions, but hardly to be recommended for routine practice. In virtually all mammalian species (hystricomorphs, which are very precocious at birth, are a possible exception) lactation performs a crucial role in safeguarding the reproductive investment during the early stages of exterogestate life. Short's claim (1976) that 'Lactation is the central control of reproduction' has both figurative and physiological validity.

The point is perhaps emphasized by comparing the act of suckling with the only other aspect of mammalian reproductive behaviour involving passage of fluid between two individuals – copulation. In each case, neuroendocrine control is paramount, the fluid is expelled by muscular contraction, erectile tissues facilitate the fluid transfer and the act appears to satisfy a profound psychological impulse in both individuals. That the comparison is not mere analogy is emphasized by the commonality of several features to both processes, including the presence of certain nutritive and immunoreactive constituents in both fluids and the release of oxytocin from the adult pituitary gland in response to tactile stimulation.

In this volume, the subject of lactation is discussed in two chapters. Here we are concerned with the general significance of lactation and with the physiology and biochemistry of mammary function. Chapter 11 dealt with the endocrine system as it relates to lactation and, conversely, with the effects of suckling activity on maternal reproductive status.

Research on lactation is a growing field, which has been extensively reviewed in recent years in several publications, e.g. those edited by Larson and Smith (1974–78); Mepham (1983), Neville and Neifert (1983), Peaker *et al.* (1984) and Neville and Daniel (1987). The approach adopted here will necessarily be selective. Our aim is to identify the important principles of lactational function, and to do so discursively rather than merely documentarily.

12.2 THE EVOLUTION OF LACTATION

12.2.1 GENERAL

It is estimated that there are more than 4000 different extant species of mammal, but regrettably, as natural environments are progressively destroyed, the number is declining. Mammalia are classified into three superorders: the Ornithodelphia, Marsupialia and Eutheria. There are some important differences, as well as many similarities, between the lactational processes exhibited by species of the three superorders. However, space does not permit more than a generalized approach, and this inevitably means concentration on eutherians, which constitute over 95% of all mammalian species. Readers are referred to Griffiths (1983) for a review of lactation in monotremes (Ornithodelphia) and to Renfree (Chapter 7) for a review of marsupial lactation.

The single morphological feature by possession of which mammals are classified as

distinct from other vertebrates is the mammary gland. Since lactation is a crucial element of the mammalian reproductive strategy it seems important to examine evidence relating to the evolution of the gland and its secretion. This necessitates some consideration of the physiological implications of palaeontological findings.

Mammals are thought to have evolved from synapsid (mammal-like) reptiles during the Triassic period, approximately 200 million years ago (Kemp, 1982). Palaeontological evidence suggests that all early mammals were small (about the size of shrews), homoiothermic, nocturnal animals, which fed on insects and other invertebrates. The radiation of the mammals during the Cretaceous/Tertiary transition (70–150 million years ago) coincided with the extinction of dinosaurs and other larger reptiles, and it is probable that these divergent outcomes were related to the ability of mammals to withstand much more readily the pronounced climatic and vegetational changes occurring during this period (Pond, 1976). Juvenile reptiles are morphologically similar to adults but, because the young of large species are so much smaller than their parents, their diets differ markedly. Thus, breeding populations could only become established in habitats providing food suitable for all developmental stages. It seems that this constraint, exacerbated by the inability to cope with marked temperature fluctuations, proved an insuperable problem for dinosaur survival (Pond, 1976).

By contrast, for mammals, the habitat need only satisfy the nutrient requirements of the adult, since the neonatal diet, in the form of milk, is produced at the expense of the maternal diet and/or tissues. Parental feeding of juveniles is a widespread phenomenon, being practised by species as diverse as birds, fish and insects (Chadwick, 1977), but its particular nature in mammals may be seen to confer certain 'advantages' by comparison with alternative strategies (such a claim is not meant to imply 'causal teleology', as is discussed in section 12.2.5).

Thus, within limits, irrespective of the precise nature, mass and frequency of intake of the maternal diet, the neonate receives relatively constant supplies of milk of fairly uniform composition, because milk precursors may be mobilized from maternal body stores. This metabolic strategy has several consequences favourable to survival and reproduction.

1. Freed of responsibility for its own survival, the neonate may continue to grow and develop, in 'embryonic' fashion, to attain sexual maturity, a condition reached much earlier in mammals than in reptiles of equivalent body weight.
2. Lactation frees the mother from the necessity to carry young *in utero* for prolonged periods, a factor of considerable value to species in which there is reliance on speed to escape predators or on mobility to search for food.
3. Lactation effects a substantial economy in neonatal metabolism by allowing a delay in the appearance of teeth until the skull and jaws are almost fully developed: mammals exhibit diphyodonty (two sets of teeth) in contrast to the polyphyodonty which characterizes reptiles (Pond, 1976).
4. Finally, lactation would appear to exert a crucial role in the establishment of social organization, a factor of undoubted importance in species survival. The sustained physical proximity between adult and young, on which nursing depends, provides a psychological environment conducive to cooperativity and learning, which in our own species has resulted in all those achievements of art and culture which are valued so highly. As remarked elsewhere, 'perhaps not without justification we might perceive the human breast, innocently elaborating a seemingly bland fluid, to be the very font of human civilization' (Mepham, 1987a).

These distinctive features of the lactational process seem highly suited to a 'colonizing' strategy, i.e. the practice by which animals may reproduce rapidly in a highly changing environment. By analogy with weeds, mammals were able to establish themselves in habitats inhospitable to those species, like the large reptiles, with more fastidious ecological demands.

12.2.2 THE EMERGENCE OF MAMMARY FUNCTION

Most theories of the evolution of lactation have regarded it primarily in terms of mammary development. The theory of Darwin (1872), as modified by Long (1972), adopts the following scenario: (i) the development in therapsid reptiles of a vascularized incubation area ('brood patch') following the attainment of homeothermy; (ii) enhanced neonatal survival through utilization of the cutaneous secretion from the vascularized area; (iii) utilization of the secretion as food following chance ingestion by the neonate; (iv) improvements in suckling behaviour, transfer processes (e.g. teat elongation) and nutritional quality of the secretion.

Several suggestions have been made for the primary adaptive benefit of the protolacteal secretion (stage ii, above). For example, Bresslau (1920) proposed a role in heat transfer; Haldane (1965) suggested maintenance of water balance by prevention of desiccation; and Graves and Duvall (1983) invoked the theory that the ventral exudates possessed pheromonal properties which facilitated maternal–infant aggregation. One recent theory is particularly attractive in that it draws on the molecular evolution of a pivotal factor in mammary biosynthetic processes, α-lactalbumin, which is a component of the enzyme lactose synthetase (section 12.6.3). It was first proposed by Brew and colleagues (see Brew, 1970), and more recently confirmed by extensive analyses (e.g. Phillips *et al.*, 1987), that α-lactalbumin was most probably derived from lysozyme by a process of gene duplication. Hayssen and Blackburn (1985) interpreted such findings as indicating that the primary role of protolacteal secretion was that of protecting the eggs or young from bacterial, fungal or protozoal infection. It is interesting that while a lysozyme with bactericidal activity has been detected in both placental and monotreme (i.e. echidna) milks, the milk of echidna does not contain α-lactalbumin. The lysozyme of echidna milk does, however, show activity in the lactose synthetase system (Hopper and McKenzie, 1974). It thus appears that monotreme milk contains a protein which is a structural and functional intermediate of lysozyme and α-lactalbumin. Hayssen and Blackburn (1985) envisaged that duplication of the lysozyme genetic material may have occurred as early as 300 million years ago. Thereafter, as the duplicated material evolved, its product became involved in milk secretion in an intermediate form, possessing both nutritive and lysosomal activity, as in echidna milk. According to this theory, although the gene for lysozyme duplicated much earlier, the evolution of α-lactalbumin only occurred after the split between monotreme and placental species. In fact, lysozyme is only one of several microbial inhibitors in milk, including, for example, immunoglobulins, lactoferrin, peroxidase and xanthine oxidase, which suggests that the protective role of milk predated its nutritional function (Blackburn *et al.*, 1989). The protolacteal secretion might have provided important antimicrobial protection at the egg surface and/or, following ingestion, in the pharynx and digestive tract of the offspring.

This postulated link between bactericidal and nutritive functions of milk provides a rationale for the evolution of milk which seems appropriately parsimonious. But the question of the evolution of suckling is more problematical. The issue was clearly identified by Mivart in the following terms (cited by Darwin, 1872).

> Is it conceivable that the young of any animal was ever saved from destruction by accidentally sucking a drop of scarcely nutritious fluid from an accidentally hypertrophied cutaneous gland of its mother? And even if one was so, what chance was there of the perpetuation of such a variation?

Haldane (1965) addressed these questions and concluded that suckling behaviour was not accidental, but an aspect of infant behaviour developed prior to the emergence of the nutritive secretion. This reasoning, articulated with characteristic wit and erudition, rests on analogy with behaviour patterns of certain extant birds in hot, dry climates, e.g. lapwing and sand grouse. Such birds periodically wet their feathers by water immersion and then sit on their eggs, thus cooling them. Had this practice been adopted by therapsid reptiles in hot climates not only would hatching rates have improved, but the wet hair would also have provided the newly hatched young with a necessary water supply, a consideration of even greater importance had these species been ureotelic. Haldane envisaged that both habits, parental hair-wetting and hair-sucking by the young, became genetically determined so that when milk evolved the young were already equipped to exploit this novel source of water and nutrients.

Attractive as the theory may seem, it assumes that mammals evolved from hair-coated reptiles in hot climates, an assumption challenged by Long (1972) but supported by Kemp (1982). Recently, reviewing a wide range of evidence, Blackburn *et al.* (1989) have deduced that lactation probably arose in endothermic, oviparous ancestors, possessing hair (probably a pelage) and exhibiting a degree of maternal care. Behavioural and psychosexual aspects of suckling are discussed by Blass and Teicher (1980).

12.2.3 THE EVOLUTION OF LACTATIONAL METABOLIC CAPACITY

It was for long considered that the mammary gland evolved from eccrine sweat glands, but a range of evidence now suggests closer homology with epitrichial (apocrine and sebaceous) glands. However, since the two types of epitrichial glands have specializations which preclude their direct ancestry to the mammary gland, it has been suggested that the latter either arose from an evolutionary precursor of extant epitrichial glands or represents a neomorphic 'hybrid' assemblage of the two gland populations (Blackburn *et al.*, 1989).

According to this scenario the mammary gland acquired its specific nutritive role through amplification of the secretory activity of these premammalian cutaneous glands. But it is questionable whether such changes were of a qualitative nature. Thus, certain extant lepidosaur reptiles possess lipid-secreting glands, which, like mammary glands, are sensitive to endocrine influences, including that of prolactin (see Pond, 1984). If the appearance of the mammary gland were to mark the emergence of mammals, one might have to conclude that it represented merely a quantitative change in a pre-existing morphological structure. Indeed, Van Valen (1960) has argued the case for inclusion of therapsids in the class Mammalia.

There is, however, another aspect of lactation which merits attention, namely the implications for maternal metabolic activity. Kemp (1982) identified three major problems for land-dwelling animals, namely temperature variation; loss of body water, with attendant difficulties in maintaining osmotic and ionic balance; and gravitational problems due to the absence of buoyancy in air. Successful mastery of such problems in mammals has largely depended on endothermy, an efficient, but in energy terms, extravagant, means of achieving homeothermy. However, the problems faced by juveniles are poten-

tially more severe than for adults because not only is energy required for growth but their greater 'surface area–volume' ratio also renders them more subject to temperature variation and water loss. Thus any strategy for mammalian neonatal nutrition would seem to depend on a high rate of maternal nutrients and, particularly, energy provision in milk. The scale of energy transfer involved is indicated by Blaxter's (1961) calculation that for certain small mammals (which all mammals were throughout the Mesozoic era; Lillegraven, 1980) the energy content of the milk secreted in a single day is equal to that of the whole litter at birth. It seems clear that so abrupt and major a metabolic change could only be achieved by maternal acquisition of the capacity to accumulate and mobilize energy and nutrient supplies adequate to sustain milk formation: and it is for this reason that Pond (1983, 1984) has argued that the significant quantum change in mammalian evolution was metabolic rather than morphological.

In most mammalian species, fat (with an energy value per gram twice that of both protein and carbohydrate) is the main form of milk energy. Pond's analysis of adipose tissue in several species has revealed a relatively uniform interspecies distribution of adipocytes of similar size, which may signify that the organization of adipose tissue evolved principally in connection with its role in provisioning lactation (Pond, 1984). In any event, in many species lactation is attended by a profound reorientation of maternal metabolism such that nutrients are preferentially channelled to the mammary glands. This recasting of metabolic priorities, which has been termed 'homeorhesis' (see Bauman and Elliot, 1983), is largely regulated by endocrine factors. In high-yielding dairy cows, it is signified by a negative maternal energy balance for about 10 weeks post partum, with approximately 30% of milk energy being derived from mobilization of body fat over the first month (Bauman and Elliot, 1983).

This analysis suggests the following scenario, which is based largely on that of Blackburn *et al.* (1989).

1. Evolution of egg incubation behaviour in synapsid reptiles, following development of hair, endothermy and cutaneous glands;
2. evolution of a well-vascularized incubation patch on the ventral abdomen;
3. enhancement of egg survival by the antimicrobial action of cutaneous gland secretions of the incubation patch;
4. hypertrophy of these cutaneous glands, leading to their increased secretory activity, probably in response to stimulation by reproductive hormones;
5. gradual changes in the composition of the cutaneous glands' secretion to produce a more nutritious product, which first supplemented and subsequently replaced yolk nutrient provision;
6. concomitant increases in milk nutrient content and transfer efficiency to the young (e.g. suckling, milk ejection) and, following divergence of the ancestors of extant mammals, evolution of specializations, such as teats.

12.2.4 MAMMARY GLAND NUMBERS AND LITTER SIZE

An obvious limitation of lactational capacity of evolutionary significance is mammary gland number. It is common knowledge that the number of glands, and their distribution, is highly species variable (section 12.3). Aristotle (third century BC) was probably the first to record the claim that a species typical litter size is positively correlated with gland number, but in modern times the assertion has been questioned (e.g. Anderson and Sinha, 1972). The issue has recently been extensively examined for the class Rodentia by Gilbert (1986). Data from 266 species showed that mean litter size and gland number are significantly and positively correlated accord-

ing to a 'one-half rule', i.e. on average rodents have litter sizes equal to half their gland number. An apparent anomaly proved, on closer inspection, to add a new dimension to the analysis: arboreal squirrels, unlike terrestrial forms, appeared to underutilize their mammary capacity – until it was realized that the former were capable of gestating and nursing two litters simultaneously. However, a curious observation provides a salutary warning against too summary an analytical approach. Horseshoe bats (Rhinolophoidea) have two pairs of teats, but the pair located near the back legs, which are very large and jut out of the fur, serve no secretory role but merely act as 'dummies' – attachment points for the young that facilitate their transport by the mother during the first days after birth (Wickler, 1969). This strategy is also employed by certain marsupial and rodent species to transport the litter from the nest in times of danger (Blass and Teicher, 1980).

Given the association between gland number and litter size, it is pertinent to consider whether the former limits the latter or vice versa. Clearly, in a contemporary context, the mother can only nurse simultaneously a litter size which does not exceed gland number, and there is evidence that, despite 'neonatal rotation', infant mortality increases when this number is exceeded. Indeed, Gilbert's (1986) analysis showed that maximum litter size was almost identical to gland number. But this does not reveal the causal relationship on an evolutionary time-scale. However, while it is possible by selective breeding to substantially increase litter size in a few generations, attempts to breed for supernumery glands have had only limited success. Thus, it appears more likely that it is gland number which has determined litter size rather than the reverse.

12.2.5 WHY DON'T MALES LACTATE?

In concluding this brief survey of the evolution of lactation, it is appropriate to consider an apparent constraint on the adoption of this reproductive strategy. Pigeons and doves exhibit a form of parental care analogous to lactation in which parents of both sexes regurgitate so-called 'crop milk' and feed it to their newly hatched squabs. The fact that both parents produce crop milk raises the question of why this does not appear to have been reported for mammals. It is clearly a matter of general interest: Gould (1987), essayist and popularizer of biology, has revealed that no single item has aroused more curiosity among his correspondents than 'why men have nipples'.

Daly (1979) has argued that, given the long interval between conception and parturition, it is unsurprising that male parental investment of any kind should be slight. Generally, low paternity confidence, i.e. the probability that the male sired the offspring in question, would seem to be decisive. There are, however, some species in which stable pair bonding, with consequent high paternity confidence, is the rule. Daly's analysis of data for two such orders of mammals, the Canidae and the Hylobatidae, revealed that male parental investment was significant but took forms such as provisioning the mother with food and protecting the territory. In the case of canids, lactational capacity does not appear to limit neonatal survival; constraints are more probably imposed by the family's capacity to derive adequate food from the available hunting grounds in the most arduous season. Daly (1979) concluded that male lactation, while not posing insurmountable physiological problems, has probably been precluded because sexually dimorphic lactational physiology had already evolved substantially before any involvement of male parental investment occurred.

12.3 MORPHOLOGY OF THE MAMMARY GLAND

12.3.1 GENERAL

Notwithstanding the claim that lactation may have evolved primarily in consequence of changes in maternal metabolism, in present-day mammals the mammary gland assumes a paramount role as both the site of milk synthesis and secretion and the sensory organ on which regulation of the whole process largely depends. At least from the time of Galen (second century AD) to that of von Haller (eighteenth century), the glands were assigned the merely passive role of filters, separating off substances preformed in blood (see Mepham, 1986). By contrast, the modern conception is that in many respects the metabolic activity of lactating mammary glands surpasses that of most other organs of the body. The object of this section is to review briefly our current understanding of the morphological infrastructure which supports and channels this activity. Mammary development is not discussed here: readers are referred to reviews by Cowie *et al.* (1980), C.W. Daniel and Silberstein (1987) and Russo and Russo (1987).

Milk is produced by the mammary epithelium, a single-cell layer arranged in numerous spherical alveoli, into the hollow lumina of which the milk is secreted (Figure 12.1).

Surrounding each alveolus is a loose network of myoepithelial cells which, by contracting, expel milk from the alveolar lumina. Groups of alveoli assume a racemose configuration, with milk from neighbouring alveoli draining into common ductules and ducts of progressively larger diameter. Groups of alveoli form lobules, which are separated from neighbouring lobules by connective tissue septa. It is thus usual to categorize parenchymal tissues as either lobuloalveolar or ductal. The ducts terminate in storage spaces beneath the teat or nipple,

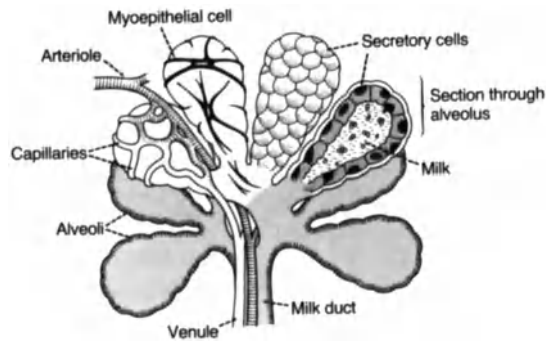


Figure 12.1 Diagrammatic representation of a cluster of mammary alveoli showing, from left to right: the outer vascular layer; within the latter, the myoepithelial cell layer; within the latter, the bases of the secretory cells; and finally, a transverse section through an alveolus.

from which milk is removed by the suckling young. The relative size of these spaces is highly species variable, e.g. in rats less than 10% of the milk may be contained therein prior to suckling, whereas for dairy animals the spaces (termed 'cisterns' or 'sinuses') may accommodate half the milk. Non-parenchymatous mammary tissues constitute the stroma, i.e. skin, blood and lymph vessels, nerves, connective and adipose tissues. Descriptions of the gross anatomy of mammary tissue of various species are referenced by Cowie and Tindal (1971) and G.H. Schmidt (1971).

Mammary gland number and disposition on the ventral body surface differ appreciably between species, e.g. two pectoral glands in humans and elephants; two inguinal glands in goats and guinea pigs; four inguinal glands in cows; and numerous glands, arranged in bilateral pairs along the abdominal surface, in rats, cats, dogs and pigs. Wherever situated, the glands share the blood, lymph and nerve supply of the contiguous skin area. Mammary arteries terminate in networks of capillaries surrounding each alveolus (Figure 12.1), but arteriovenous anastomoses and arteriovenular bridges are common in mammary tissue, particularly in the teats.

Mammary innervation is principally of two types: (i) sensory nerves originating from tactile receptors in the skin, the receptors, in the form of unmyelinated nerve endings being particularly prevalent in the teats (Cross and Findlay, 1969), and (ii) sympathetic motor nerves innervating contractile tissues of the blood vessels (which effect vasomotor control) and teat musculature. Contraction of the latter causes teat erection, thus facilitating its retention in the mouth of the suckling young. There is also electrophysiological and histochemical evidence for sparse innervation of a third type, namely of the large ducts: this might be involved both in detection of changes in intraductal pressure and, by reflex changes in smooth muscle tone, in altering ductal capacity (Linzell, 1974). Myoepithelial cells are not innervated. For more detailed discussion of mammary blood vessels and nerves the reader is referred to Linzell (1974).

12.3.2 ULTRASTRUCTURE

The functional unit of the mammary epithelium is the secretory cell. This performs the roles of absorbing materials, which have been delivered in arterial blood, from the interstitial fluid; synthesizing milk constituents; and transferring these synthesized products, together with certain others which have undergone no metabolic change, into the alveolar lumina.

The ultrastructure of the secretory cells, as revealed by electron microscopy, provides many clues as to how these processes occur (Figure 12.2).

The cells are generally roughly cuboidal,

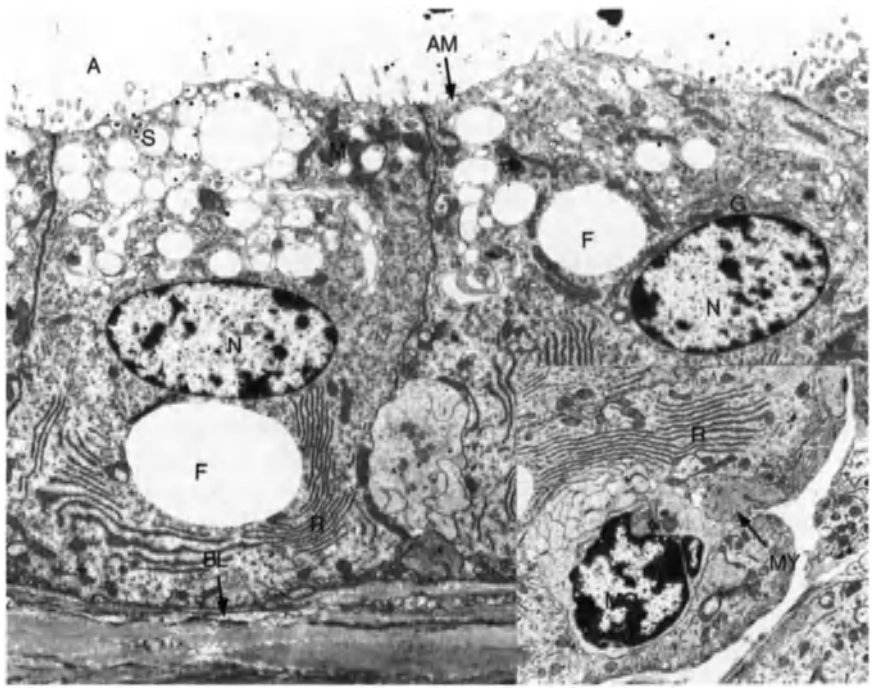


Figure 12.2 Electron micrograph of two secretory cells in bovine mammary tissue (scale bar 1.0 μm). A, alveolar lumen; AM, apical membrane; BL, basal lamina; F, fat droplets; G, Golgi apparatus; S, secretory vesicle containing protein granules; N, nucleus; R, rough endoplasmic reticulum; M, mitochondria. Insert: MO, monocyte; MY myoepithelial cells. (Courtesy of Dr F.B.P. Wooding.)

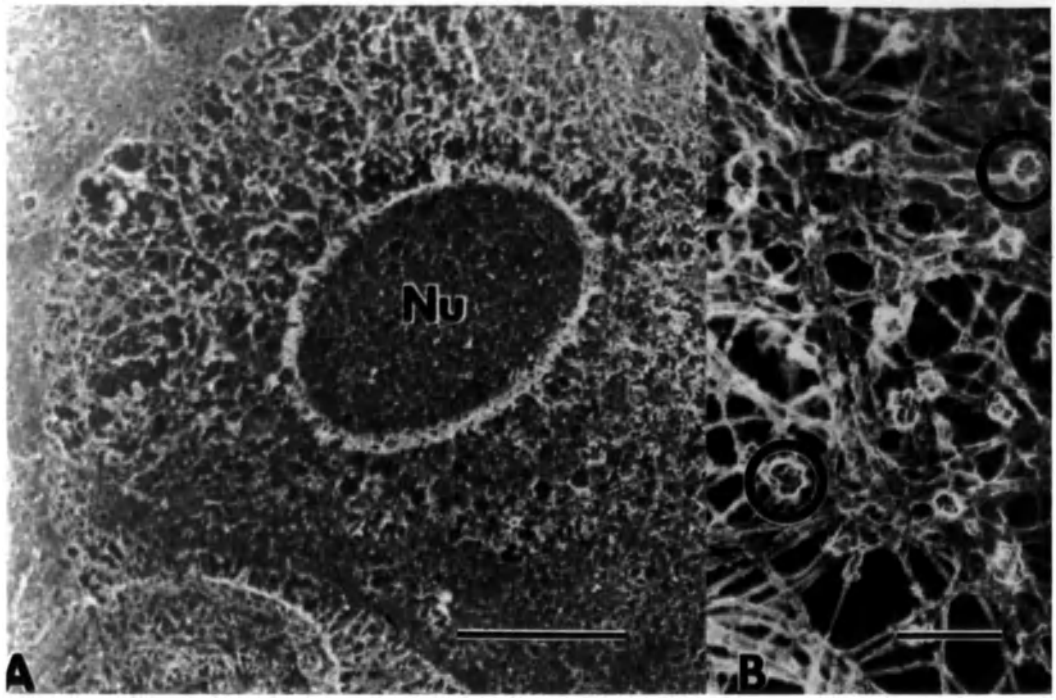


Figure 12.3 Platinum replicas of detergent-insoluble primary mouse mammary cell cytoskeletons. In A, at low magnification (scale bar 5 μm), the nuclear lamina (Nu) and cell borders are easily visualized. In B, at higher magnification (scale bar 0.2 μm), polyribosomes (circles) are attached to the filamentous cytoskeleton. Micrographs are printed as negatives (Reproduced from Aggeler *et al.*, 1988, with permission.)

but become flattened in the apicobasal axis when the alveoli are full of milk and, conversely, elongated just following milk ejection. The cytoarchitecture also shows a pronounced apicobasal polarity, i.e. the nucleus tends to be situated basally and much of the apical cytoplasm is crowded with Golgi and secretory vesicles, containing protein granules, and with fat globules. Rough endoplasmic reticulum is well developed and numerous mitochondria are scattered throughout the cytoplasm. The basal membrane is highly convoluted, with many infoldings and interdigitating processes, but by contrast the apical membrane bears only a diminutive 'hedge' of microvilli, interrupted in some sections by fat droplets in the process of 'pinching off' and secretory vesicles undergoing exocytosis (section 12.7). Despite the wide range of compositions in milks of different species, there appears to be considerable uniformity in mammary tissue at the ultrastructural level (Wooding, 1977).

The fibrillar infrastructure of all cells is composed of the cytoskeleton (Figure 12.3), a system involved in maintaining structural integrity, transport of materials within cells, cell motility and intercellular communication.

Three cytoskeletal systems have been described: microfilaments of actin (6 nm diameter); intermediate filaments of keratin (7–11 nm); and microtubules of tubulin (22 nm). In the mammary gland the constituent proteins of these systems may exist in either free or polymerized form, and lactation is associated with a marked shift in equilib-

rium towards a greater degree of tubulin polymerization, as well as with **net** tubulin synthesis. The cytoskeletal system appears crucial for milk synthesis and secretion, and it is hardly surprising that it has been shown to be influenced by several endocrine and other regulatory factors which affect milk secretion (see Loizzi, 1987). There are even suggestions that the cytoskeleton may participate in nuclear regulation of mRNA processing. To quote Bissell and Hall (1987), 'The cytoskeleton. . . . emerges as the structure most likely to integrate form into function'.

Cell junctions

Description of the properties of the secretory epithelium is, however, by no means encompassed by reference to intracellular features. Of crucial importance are the intercellular junctions which confer on the epithelium several important properties, namely mechanical strength, apicobasal polarity, coordination of physiological activity and restriction of solute leakage between cells (the paracellular route). The study of mammary cell contacts has recently provided several new insights, which are summarized briefly below.

With the introduction of electron microscopy, the junctional complex between neighbouring cells (referred to as the 'terminal bar') was seen to comprise three distinct junctions (Pitelka, 1985). Invariably, the most apical of these is the 'tight junction' (zonula occludens), a region where the outer leaflets of two cells appear to be intermittently fused (Figure 12.4).

The tight junctions form belts surrounding the apical poles of cells, so that each is sealed to its neighbours in a continuous, cellular mosaic. Below the tight junction is the intermediate junction (zonula adhaerentes) and, in many sections, beneath that are one or more desmosomes (maculae adhaerentes). In both intermediate junctions and desmosomes (Figure 12.4) a dense material is present under, and occupying the 20–30 nm space between, the two cell membranes: this intercellular 'glue' probably consists of membrane-associated glycoproteins. In addition to these three types of junction, gap junctions (maculae communicantes), 'spots' in which a 2–4 nm gap separates two apposed membranes, are scattered in apparently random fashion in tissue sections.

Research over the last 20 years has led to the ascription of specific functions to the different junctional types. Thus, tight junctions serve the roles of acting as a paracellular permeability barrier and as intramembrane barriers delimiting the apical and basal domains. Both intermediate junctions and desmosomes are adhesive, their locations serving to maintain epithelial structural integrity and to protect tight junctions from mechanical damage such as might occur when intra-alveolar pressure is raised. Desmosomes are particularly well developed in ductal tissue, where they connect ductal and epithelial tissues in like and unlike pairs, while hemidesmosomes appear to be sites of attachment of myoepithelial and epithelial cells to the basal lamina and fibrous stroma. Gap junctions act as leakproof intercellular communication channels, allowing the spread of ions, small molecules and electrical currents. They may thus provide an explanation for the synchronous secretory behaviour of cells in a single alveolus (Pitelka and Hamamoto, 1983), but paracrine secretions (section 12.8.4) crossing the apical membrane may also be involved.

The cell junctions are further characterized by the cytoskeletal elements of which they are composed. Actin filaments, continuous with a web beneath the apical membrane, attach to membranes of tight and intermediate junctions; and keratin filaments are inserted into, and looped through, the dense cytoplasm of belt and spot desmosomes. Gap junctions are composed of 7- to 8-nm-diameter protein particles (connexons),

which are arranged in roughly hexagonal fashion in linking two apposed cell membranes: each connexon is penetrated by a 2-nm-diameter central pore, which thus provides a continuous hydrophobic channel between the two cells (Pitelka and Hamamoto, 1983).

Insight into the nature of cell junctions has been obtained by use of the freeze–fracture technique, which involves freezing excised tissue in liquid nitrogen and then breaking it in two. The fracture tends to follow natural cleavage planes, thus exposing an inner protoplasmic (P) face of the leaflet and an outer exoplasmic (E) face. A metal replica for transmission electron microscopy may be prepared by deposition of a platinum–carbon film on the cleaved surface, followed by dissolution of the tissue. Use of this technique reveals the lines of fusion of two cell membranes as a network of ridges, about 8 nm in diameter, on the P face and as complementary grooves on the E face (e.g. Morgan and Wooding, 1982). The pattern is continuous where the fracture plane shifts from one membrane to the other, indicating that the ridges and grooves are closely parallel and probably make contact in the intercellular spaces. It is presumed that in living tissue the junction is consolidated by fusion of outer lipid leaflets of the junctions: the ridges themselves are thought to be proteinaceous (Pitelka, 1985).

Since tight junctions constitute the paracellular permeability barrier, much interest has centred on the ultrastructural changes they might undergo in conditions where leakage is believed to occur, e.g. prior to parturition,

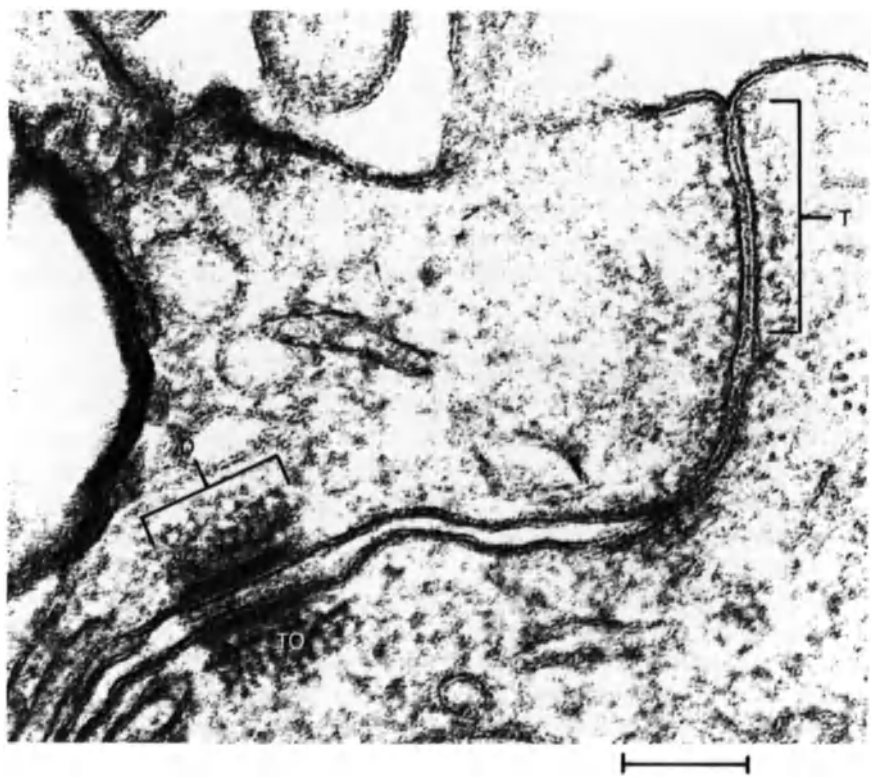


Figure 12.4 Electron micrograph of the apical regions of two secretory cells in ovine mammary tissue (scale bar 0.1 μm). T, tight junction; D, desmosome; TO, tonofilaments. (Courtesy of Dr F.B.P. Wooding.)

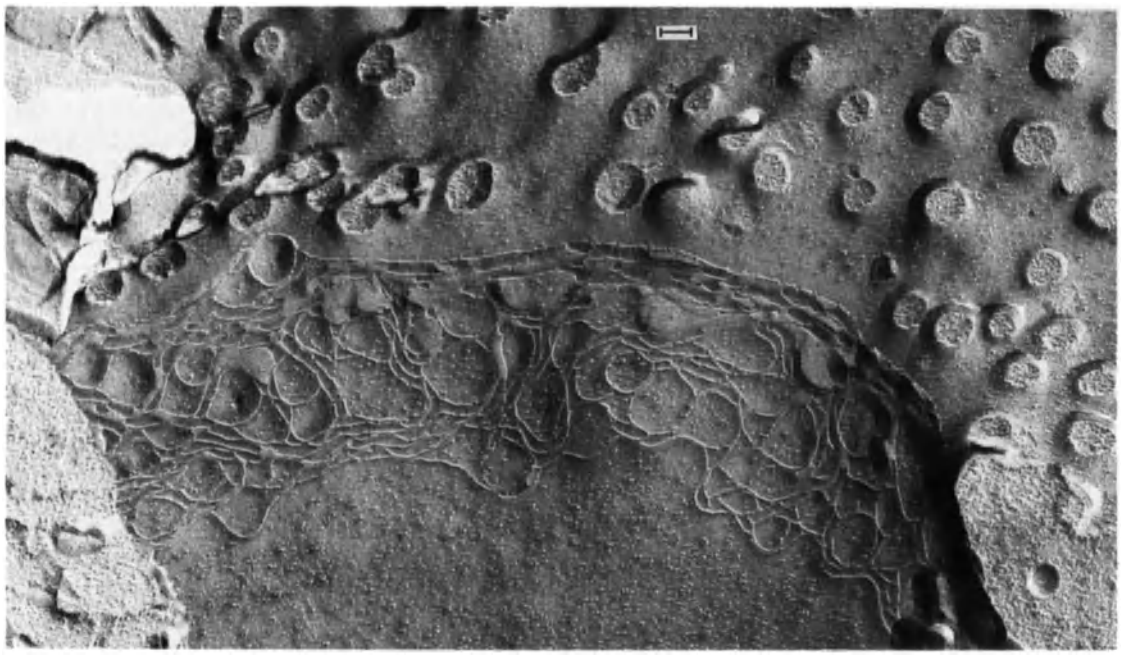


Figure 12.5 Freeze–fracture replica of part of a tight junction belt between two secretory cells in fully lactating (1 month post partum) ovine mammary tissue (scale bar 0.1 μm). (Reproduced from Morgan and Wooding, 1982, with permission.)

during the oestrous cycle and in late lactation. Freeze–fracture studies on mammary tissue from sheep (Morgan and Wooding, 1982) and mice (Pitelka and Taggart, 1983) have indicated that in such conditions the ridges become disordered and variously orientated, in contrast to the compact network, parallel to the apical border, which is characteristic of the normal lactating gland (Figure 12.5).

In rabbits, a species in which there is a pronounced paracellular leak in declining lactation, the junctional complexes become drastically attenuated a few days after attainment of peak milk yield, and it seems likely that ridge number varies inversely with degree of leakage, possibly in a logarithmic relationship (Pitelka, 1985).

The ridge pattern would appear to adapt readily to changes in cell shape in the course of the alveolar filling and emptying cycle. Thus, Pitelka and Taggart (1983), investigating the effects of mechanical tension in cells of a confluent epithelial monolayer plated on collagen gel, showed that undirectional stretching led to repositioning of the junctional ridges along lines of least resistance.

12.3.3 MYOEPITHELIAL CELLS

In many micrographs of mammary tissue it is possible to identify transverse sections of myoepithelial cells in close proximity to the basal surface of secretory cells (Figure 12.2). Their orientation, surrounding alveoli in a basketwork arrangement is, however, more readily appreciated in scanning electron micrographs (Figure 12.6).

In the rat gland, there appear to be about five cells per alveolus, each with 3–5 primary processes extending from the nuclear region, which branch to give 8–10 terminal processes per cell.

Ultrastructurally, myoepithelial cells are

similar to smooth muscle cells, the cytoplasm being replete with filamentous tracts and plasmalemmal caveoli: the tracts are composed of actin filaments and a loose network of intermediate filaments. Apart from myofilaments, the cells possess the usual organelles, but in poorly developed form, e.g. few mitochondria, a diminutive Golgi apparatus, free ribosomes and small cisternae of rough endoplasmic reticulum (Hollman, 1974).

Since myoepithelial cells contract in coordinated fashion in the milk ejection reflex, it is likely that some form of intercellular communication exists. There is general agreement that intra-alveolar cells do not form a

Figure 12.6 Scanning electron micrograph of mammary alveoli from a lactating rat, with most connective tissue removed by enzyme and acid treatment (magnification ×1000). V, small blood vessels; M, myoepithelial cells; S, secretory cells (Reproduced from Nagato *et al.*, 1980, with permission.)

syncytium, and it is surmised that gap junctions provide the route by which the spread of regulatory ions or molecules is effected (Pitelka and Hamamoto, 1983).

12.3.4 EXTRACELLULAR MATRIX

A recent area of research, which seems likely to prove extremely fruitful, is that concerned with the role of the extracellular matrix, the complex of acellular material by which cells are connected within tissues. In epithelial tissues this includes the basement membrane, which contains the basal lamina (derived from parenchymal cells) and a reticular lamina (derived from connective tissue cells), the two acting as a functional unit (see Bissell and Hall, 1987). In mammary tissue the basal lamina is an approximately 30-nm-thick, low-density layer, which forms a smooth blanket around the alveolar epithelium (including the myoepithelial cells) and is composed of a specific non-fibrillar collagen type IV, a glycoprotein (laminin) and other glycoproteins and glycosaminoglycans (see Pitelka and Hamamoto, 1983). Not only does the extracellular matrix influence the form of the epithelial cells and the development of the mammary epthelium as a whole, but it also interacts with the secretory cells during lactation, modulating their synthetic and secretory activity. Properties of the extracellular matrix have been investigated by using primary cell cultures in defined media on various substrata, from which it has been demonstrated that, for example, markedly different patterns of protein are secreted when cells are cultured on plastic, on attached collagen gels and on released collagen gels. In some cases proteins which are not normally present in milk are secreted, suggesting that alteration of the extracellular matrix may remove repressors or induce promoters. Many of these effects may be mediated by changes in cell morphology and cytoarchitecture (Bissell and Hall, 1987).

12.4 MILK COMPOSITION

12.4.1 GENERAL

Analyses of the compositions of milk of different species reveal the striking fact that, apart from superficial qualitative similarities, there is an enormous variation in the concentrations of specific constituents. There can be little doubt that evolutionary pressures have been paramount in shaping these compositional differences, but the notion that each milk is ideally suited to the 'needs' of the young (a notion which finds frequent expression, even in scientific literature) comes uncomfortably close to the causal teleology (aptly nicknamed Panglossism, with reference to Dr Pangloss in Voltaire's *Candide*), which has been so rightly deprecated by Medawar and Medawar (1984). How the infant develops is clearly, in some measure, a consequence of what sort of, and how much, nutrient it receives: its appetite is only one side of the equation. Moreover, a simplistic 'nutritional' rationale for milk composition might be misleading because milk contains not only recognized nutrients but also chemicals whose presence might best be described as 'adventitious'. A case in point is the regrettable appearance of polychlorinated biphenyls (PCBs), from pesticide use, in milk (Rogan, 1986). How many milk constituents fall into this category of useless and/or harmful components?

However, even allowing for the importance of evolutionary pressures in shaping milk composition, it is evident that factors such as physicochemical necessity and maternal metabolic capacity must constitute important constraints. Wilson (1975) discussed the latter aspect in terms of 'parent–offspring conflict', a theme provocatively espoused by Dugdale (1986), who claimed that human milk is unlikely to be optimal for infant development, as to secrete such a milk would jeopardize maternal survival. Latham (1986) would appear to have effectively countered

this claim since, even ignoring a whole range of attributes of lactation which benefit both mother and baby, the difference in maternal energy cost between 'optimal' and 'adequate' energy supply to the infant seems insignificant in humans. Moreover, from their extensive studies, Prentice and Whitehead (1987) concluded that 'human pregnancy and lactation are remarkably resilient to the effects of energy restriction'. Nevertheless, there must come a point when the costs to the mother of her parental investment outweigh the perceived benefits (however 'cost' and 'benefit' are defined) and this point may be reached earlier in species in which lactation imposes a proportionately greater strain on maternal metabolism, such as small laboratory animals, than it usually does in women.

12.4.2 GENERAL CHARACTERISTICS OF MILK

It would be inappropriate in a chapter of this nature to present a detailed survey of milk composition. Readers are referred for such information to Davies *et al.* (1983), Jenness (1985) and the books edited by Fox (1982, 1983, 1985). Rather, the object of this section is to survey briefly the important functional aspects of milk composition and the physiological 'strategies' which have resulted in the wide spectrum of milk compositions reported.

It is usual to discuss milk constituents according to major nutrient types, such as carbohydrates and proteins, and this approach is adopted below. From a functional viewpoint, however, it also seems appropriate to employ three categories, namely (i) nutritional (i.e. macronutrients), (ii) protective (immunological and other antimicrobial properties) and (iii) regulatory (i.e. including hormones, growth factors, enzymes, vitamins and trace elements). The categories are not absolute since some milk components perform several functions. The properties of the different milk constituents in relation to these three functions are discussed in turn.

One or two general points should be noted at the outset. Citations of data on milk composition are biased to cow's and human milk. This is because these milks have been examined more rigorously than others, because they are of greatest practical importance for most people and because they exemplify quite well the scale of interspecies variation. Most values quoted, for these and other milks, are for 'mature' milk, i.e. around peak lactation, but marked changes occur during the course of lactation. Two examples will illustrate this point: (i) in most species, protein concentrations are high, due to the presence of immunoglobins, in the period immediately following parturition, when the secretion is termed colostrum; (ii) in rabbits the lactose concentration falls 80% between days 20 and 30 of lactation (Cowie, 1969).

12.4.3 NUTRITIONAL PROPERTIES OF MILK

Lipids

Milk lipids are a chemically heterogeneous group of compounds, which are most usually referred to as 'milk fat', although the terms are not strictly synonymous. They are the most variable milk components, in both concentration and chemical composition, whether interspecies, intraspecies or within-animal differences are considered. Recorded values range from over 500 g/l in milk of some seals to trace amounts only in rhinoceros milk (see Table 12.1).

In all species, triacylglycerols (TGs) are predominant, but there is much variation depending on the relative preponderance of a wide range of fatty acids which become esterified with glycerol. For example, 437 different fatty acids have been isolated from cow's milk (Patton and Jensen, 1976). These include: normal straight-chain fatty acids of chain length 2:0 to 28:0, including all odd-carbon numbers; saturated branch-chain acids;

Table 12.1 Macronutrients in mid-lactation milks of 20 species, expressed as percentages of total dry matter mass and energy content (Adapted from Oftedal, 1984)

Species	*Dry matter as percentage of milk mass*	*Percentage of dry matter mass*				*Percentage of dry matter gross energy*		
		Fat	*Protein*	*Sugar*	*Ash*	*Fat*	*Protein*	*Sugar*
Rodentia								
Brown rat	22.1	40	37	17	5	56	33	10
House mouse	29.3	45	31	10	5	65	29	6
Guinea pig	17.5	33	36	28	5	48	34	18
Lagomorpha								
Rabbit	31.2	49	32	6	6	68	29	3
Carnivora								
Domestic dog	22.7	41	33	17	4	62	32	6
Brown bear	33.6	55	25	7	4	74	22	4
Northern fur seal	61.0	81	16	0.2	0.8	88	11	0.1
Harp seal	57.7	82	16	0.2	1	89	11	0.1
Perissodactyla								
Horse	10.5	12	18	66	4	23	22	54
Black rhinoceros	8.8	2	12	75	3	5	18	75
Artiodactyla								
Cow	12.4	30	26	37	6	48	26	26
Sheep	18.2	39	23	27	4	59	22	18
Goat	12.0	32	22	39	7	50	22	27
Pig	20.1	41	23	25	4	61	22	16
Ibex	23.3	53	24	19	5	70	20	11
Red deer	21.1	40	34	21	7	56	30	13
Reindeer	26.3	41	34	13	5	60	32	8
Water buffalo	16.8	39	24	29	5	70	20	11
Primates								
Baboon	14.6	33	9	55	2	52	9	38
Human	12.4	33	7	55	2	54	7	39

monoenoic acids 10:1 to 26:1 (including various isomers); dienoic acids of chain length 14, 16, 18, 20 and 24 (including various isomers); polyenoic acids; keto acids; and hydroxy acids (Jenness, 1974). In those species subjected to detailed study, 14 fatty acids predominate, but individual analyses may show appreciable differences depending on factors such as diet and stage of lactation. The distribution of fatty acids between the three positions of the triacyl-*sn*-glycerols is not random, an observation which appears to have physiological significance. For example, the high concentration of 16:0 in the *sn*-2 position of human milk fat renders it more digestible by the human infant than are other natural fats (Filer *et al.*, 1969).

Milk lipid is secreted in the form of spheri-

cal globules in which TG is surrounded by a milk fat globule membrane (MFGM) composed chiefly of phospholipid and protein complexes, but also incorporating cholesterol and several enzymes. In cow's milk the globules range from 0.1 to 20 μm in diameter: there appear to be species- and breed-specific distributions of globule size (see Davies *et al.*, 1983).

Proteins

Milk protein concentrations range from less than 10 g/l in primates to over 200 g/l in some lagomorphs (see Table 12.1). The two major groups of protein are caseins, which are mostly precipitated at acid pH (pH 4.6 for cow's milk) and by the action of the gastric enzyme chymosin (rennin), and milk serum (whey) proteins, which usually remain in solution at the pH of casein precipitation. Minor protein groups include enzymes and those in the MFGM.

Caseins are milk- and species-specific phosphoproteins, usually of molecular weights in excess of 20 000, in which virtually all the phosphate is combined with seryl residues as phosphomonoesters. A high concentration of prolyl residues distributed widely along the polypeptide chains gives caseins an open tertiary structure, susceptible to proteolytic digestion. Caseins are relatively hydrophobic proteins, which under the ionic conditions in milk associate in colloidal micelles of 100–300 nm diameter, composed of submicelles of 15–20 nm diameter (see Davies *et al.*, 1983).

Electrophoretic techniques indicate that casein is a complex and variable mixture of proteins. Thus, in cow's milk, α_s-, β- and κ-caseins have been described, but even such types contain different molecular forms, e.g. there are four genetic variants of α_{s1} and two of α_{s2}: the extent of phosphorylation is also subject to variation. In cow's milk, α_{s1}-, α_{s2}-, β- and κ-caseins are present in the micelle in roughly the ratio 3:1:3:1, but about 10% of the micelle consists of calcium and inorganic phosphorus, together with smaller amounts of citrate and magnesium. About 75% of milk calcium is combined directly with inorganic phosphate in micelles, the rest being bound directly to the caseins and to citrate (see D.G. Schmidt, 1982). An important 'design feature' of the casein micelle is its ability to maintain a high concentration of both protein and calcium phosphate in a dispersed form of low viscosity. This property, which is likely to be important in a fluid stored for long periods after secretion and expelled through fine-bore ducts, facilitates transfer of bone-forming minerals to the young. 'Kappa' caseins, which are glycoproteins, stabilize casein micelles, but lose this property when specifically cleaved by chymosin: the resulting curd seems to be important to effective digestion and nutrient absorption in the neonatal intestinal tract.

There is much dissimilarity between cow's and human milk with respect to casein content. Caseins constitute about 80% of cow's milk proteins, but only about 45% of those in human milk (Harzer and Haschke, 1989). Moreover, β-casein is the major casein of human milk, but α_{s1}-casein that of cow's milk. There are consequently substantial differences in the mineral contents, amino acid contents and types of curd formation in the two milks (see Hambraeus, 1982).

Serum proteins include both milk-specific (e.g. α-lactalbumin, β-lactoglobulin) and non-specific proteins (e.g. immunoglobulins and blood serum albumin). α-Lactalbumin has been detected in all milks which also contain lactose, but not in some (e.g. of the Californian sea lion) reported to be devoid of this disaccharide (see Jenness, 1974). Such differences are explicable in terms of the role of α-lactalbumin in lactose synthesis (section 12.6.3). β-Lactoglobulins are the most prevalent milk serum proteins in ruminant milks, but absent, or present at very low concentrations, in other milks (e.g. human). It has been speculated that β-lactoglobulin may

play regulatory roles in mammary phosphorus metabolism (Farrell and Thompson, 1971) and in vitamin A transport (Papiz *et al.*, 1986; Godovac-Zimmerman and Braunitzer, 1987).

Lactoferrin and transferrin are two milk proteins which bind iron. Transferrin, identical to the protein in blood, has been isolated from cow's and rabbit milk, and lactoferrin (which also occurs in secretions such as saliva and semen) from some (including human and ruminant milks) but not others (see Jenness, 1974). Relationships between the molecular structure and function of lactoferrin have been discussed by Baker *et al.* (1987). Ceruloplasmin, which binds copper, has been detected in human and cow's milk (see Jenness, 1979).

Non-protein nitrogenous compounds

Certain milks contain appreciable amounts of non-protein nitrogenous compounds, and it seems likely that some, at least, fulfil specific nutritional functions. For example, in human milk they constitute about 25% of the total nitrogen, whereas in cow's milk the figure is only about 6% (Hambraeus, 1982). Urea is generally a major component of this fraction, but in human milk taurine is present at high concentration, an observation which has been related to its possible roles in neonatal brain development (Huxtable and Lippincott, 1983), membrane protection, cell volume regulation and detoxification (Chesney, 1988).

Carbohydrates

The great majority of milks analysed contain lactose: reports of failure to detect it in some milks (e.g. of seals, sea lion and walrus) are countered by others which cite trace amounts (see Jenness, 1974). In the majority of species, its concentration is within the range 10–100 g/l (see Table 12.1), values for human milk being among the highest recorded (Jenness, 1986).

Lactose

Lactose is a disaccharide, comprising glucose and galactose linked $\beta 1 \rightarrow 4$, which is formed by the enzymic galactosylation of free glucose. Although lactose is the classical 'milk' sugar, the trisaccharide sialyllactose is a prominent sugar in the milk of rats and mice (Kuhn, 1972), and a predominant one in the milk of monotremes (see Davies *et al.*, 1983; Amano *et al.*, 1985). In human milk and cow's colostrum small amounts of lactose are modified by the addition of further sugars to form trioses, tetraoses, pentaoses, etc. The particular sugar structures so formed in human milk correspond to those on erythrocyte surfaces that define particular blood groups, since the same sets of biosynthetic enzymes are involved. Human milk contains 10–25 g/l of oligosaccharides other than lactose: cow's milk contains only 1–2 g/l.

Lactose is hydrolysed by lactase (β-galactosidase) in the neonatal intestinal brush border, and the monosaccharides released are absorbed across the gut wall into the portal blood. Galactose is converted to glucose so that it can supplement glucose as a source of metabolizable energy.

Milk salts

These exist in milk as (i) diffusible salts, i.e. low molecular weight ions and complexes, and (ii) non-diffusible salts, i.e. bound to protein. In cow's milk, the most prevalent cations are K^+, Na^+, Ca^{2+} and Mg^{2+}; the most common anions, Cl^-, inorganic phosphates and proteins. Measured as 'ash' (see Table 12.1), the mixture of incombustible mineral oxides remaining after the milk incineration, they are present in different milks within the range 2–20 g/l (Jenness, 1986). The principal ions of the non-diffusible phase are Ca^{2+}, Mg^{2+}, inorganic phosphate and citrate,

which are almost entirely associated with casein micelles. K^+, Na^+ and Cl^- are, by contrast, almost exclusively diffusible. Appreciable species differences exist in both total salt concentrations and partitioning between the diffusible and colloidal phases. For example, concentrations of the following in human milk are expressed as a percentage of their concentration in cow's milk: K, 39%; Na, 26%; Ca, 27%; P, 12%; Fe, 33%; Cu, 285%.

12.4.4 PROTECTIVE PROPERTIES OF MILK

The attainment of independent existence by the neonate is by no means as consummate as the act of parturition might appear to signify. Not only is there continuing dependence on milk nutrients, but there is also heavy reliance on parental protection from predators, parasites, bacteria and viruses. At birth, the mammalian immune system remains underdeveloped. Whereas in most species the cell-mediated (T-cell) system develops early in fetal life, it is largely ineffective because it is unchallenged in a uterine environment which admits few infectious agents. On the other hand, the humoral immune system develops later, often after birth, so that the onus for neonatal protection in the period immediately post partum continues to rest with the mother (Solari and Kraehenbuhl, 1987).

Ehrlich, in 1902, was reputedly the first to propose the transfer of passive immunity to offspring via milk (see Reiter, 1985a), but only very recently has it become appreciated how wide is the range of protective mechanisms involved. These may be summarized as: (i) selective secretion of antibodies derived from maternal blood plasma; (ii) antibody production in the mammary gland in response to local antigenic stimulation, with subsequent secretion in milk; (iii) transfer in milk of immunocompetent and phagocytic cells; (iv) secretion of non-immunoglobulin protective proteins; (v) secretion of antimicrobial lipids; and (vi) secretion of nitrogen-containing and other saccharides, which constitute the 'bifidus factor' promoting resistance to neonatal intestinal infections. Each protective mechanism is discussed briefly below. Typically, the secretion of colostrum, in which the activity of protective mechanisms is much higher than in mature milk, lasts for a relatively short time post partum. However, low levels of antibody persist throughout lactation and increase at involution, when they presumably provide resistance to mammary infections in conditions of milk stasis (Lascelles and Lee, 1978).

Passive transfer of immunity

In some species (e.g. primates, rabbits) all IgG immunoglobins are transferred to the fetus across the placental membranes, so that the neonate acquires plasma concentrations of IgGs equal to those of its mother. In other species (e.g. ungulates) all maternal IgGs are transferred in colostrum during the first few hours post partum, whereas in yet others (e.g. rodents, cats, dogs) they are acquired both *in utero* and via colostrum (see Butler, 1974). As a generalization, IgG is at highest concentration in species in which colostrum is the exclusive route, IgA in other species. Colostrum possesses several features which protect immunoglobins from denaturation and digestion in the neonatal gut, e.g. high buffering capacity, the presence of a trypsin inhibitor and, for IgG1, considerable resistance to proteolytic digestion. In ruminants, absorption of Igs into blood is limited to a period of 24–36 h post partum when the gut wall remains permeable, but there is much interspecies variation e.g. this period is 16–20 days in rodents (Butler, 1974).

Despite the short period during which Ig absorption occurs, the resistance it provides to various viral and bacterial pathogens is crucial. The protection afforded against the potent gut bacterium *Escherichia coli* suggests

that absorption across the gut wall is not always essential (Roy and Smith, 1987).

Local production of antibodies

Failure to penetrate the gut wall is, indeed, a characteristic of secretory IgA (sIgA), the principal Ig of human and rat colostrum and milk. IgAs may either be derived from maternal plasma or produced locally by plasma cells adjacent to mammary epithelial cells (Figure 12.2). In the latter case there is evidence that they are produced in response to antigenic stimuli arising during the act of suckling (Lascelles and Lee, 1978). Whether of systemic or local origin, IgA is modified by addition of a secretory component, produced by the epithelial cells, and secreted as sIgA (Solari and Kraehenbuhl, 1987).

Transfer in milk of immunocompetent and phagocytic cells

The presence of cells in milk and colostrum has long been recognized. The colostral 'bodies of Donné', described in the 1840s, and thought by some to be detached epithelial cells whose subsequent lysis was important in milk secretion (see Mepham, 1986), are now known to be macrophages replete with lipid vacuoles (Head and Beer, 1978). About 90% of cells in human milk are monocytes or macrophages, which possess the usual property of phagocytosing micro-organisms: in colostrum and milk they also produce complement components (C3 and C4), lysozyme and lactoferrin.

The remaining cells are mostly lymphocytes, of which, in human colostrum, about half are T cells and about one-third B cells: the surface immunoglobulin on many of the latter is IgA (Head and Beer, 1978). In rats and mice, T cells, reactive to transplantation alloantigens, can adoptively immunize the neonate; and such observations, together with demonstrations of the *in vitro* reactivity of milk-borne T and B cells, provide strong evidence that maternal lymphocytes reactive to micro-organisms become incorporated into neonatal tissues, where they provide short-term protection.

Secretion of non-immunoglobulin protective proteins

The antimicrobial properties of lysozyme, lactoferrin, transferrin and lactoperoxidase in milk have been stressed by Reiter (1985a,b). Lactoferrin, for example, inhibits growth of coliform bacteria by chelating iron, which is necessary for their growth. The high concentration of lactoferrin in human milk means that the infant may ingest 3 g/day in early lactation.

Apart from their specific activities, several instances of synergy have been described. For example, the protoplasts produced in the lytic activity of lysozyme on *Micrococcus luteus* are strongly agglutinated by lactoferrin; and sIgA enhances the antimicrobial activity of lactoperoxidase against *Streptococcos mutans* (see Reiter, 1985a).

Secretion of antimicrobial lipids

In recent years there has been growing evidence that milk lipids may perform a protective role. It has long been known that certain fatty acids possess antibacterial activity, e.g. 12:0, 16:1 and 18:2, but benefits only seem realizable for the suckling young when there is a sufficiently high activity of milk lipase to supplement the low intestinal activity of this enzyme. Unlike cow's milk, human (and gorilla) milk contains a bile salt-stimulated lipase (Hernell and Blackberg, 1985) which, activated in the neonatal intestine, releases fatty acids and monoacylglycerols able to effect antimicrobial action (Kabara, 1980). Host resistance applies not only to bacteria, but also to yeasts, fungi and viruses.

Secretion of bifidus factor

György (1953) was the first to describe a factor in human milk (with activity exceeding

that of cow's milk by up to 100-fold) which promotes the growth of *Lactobacillus bifidus* in the neonatal intestine. On hydrolysis the factor yielded *N*-acetyl glucosamine, fucose and galactose. Bifidobacteria metabolize milk saccharides to lactic and other acids, the resulting low pH inhibiting growth of many enteropathic bacteria. Thus, one week post partum, 95% of the culturable bacteria in the fecal smear of breast-fed infants are *L. bifidus* flora (Vorherr, 1978).

12.4.5 REGULATORY PROPERTIES OF MILK

Limitations of a classification based on functional attributes of milk constituents are, perhaps, here most evident. Components in this class include somewhat heterogeneous groups, best characterized as having important roles which are not usually defined as nutritional or protective, and which are mostly present at low concentrations. The majority of analyses have been performed on cow's and human milks.

Hormones and growth factors

Milks of several species contain factors which stimulate both growth of gastrointestinal tissues *in vivo* and replication of cells cultured *in vitro* (Koldovsky, 1989). For example, several hormones which have growth promoting action in the neonate have been detected in human milk, including insulin, thyroxine, cortisol and luteinizing hormone-releasing hormone (LHRH) (Morriss, 1985). It is possible that some of the growth effects may be additional, or subsequent, to the roles of hormones, e.g. stimulation of neonatal gonadotrophin release in the case of LHRH. But, in addition, several distinct growth factors have been described. In human milk, the following have been reported: human milk growth factors (HMGF) I, II and III; mammary-derived growth factor; transforming growth factors (Morriss, 1985); and IGF1 (Corps *et al.*, 1988). Several lines of evidence suggest that HMGF III, a 6000 Da molecular weight peptide which accounts for 75–90% of growth factor activity in the milk, is probably a form of epidermal growth factor (EGF) (Shing *et al.*, 1985; and see Dembinski and Shiu, 1987). In addition to its nutritional and protective function (section 12.6.3), lactoferrin may also have a role as a mitogenic agent for enterocytes of the suckling young (Nichols *et al.*, 1987).

Recent reports have established the presence of opioids in human and cow's milk, in both cases derived from β-casein. These oligopeptide casomorphines may influence gastrointestinal motility in the suckling young (H. Daniel *et al.*, 1990), and since they are absorbed into the blood they may also have important effects on neonatal metabolism and behaviour via the central nervous system (Teschemacher, 1987).

Enzymes

Approximately 60 enzymes have been detected in both cow's milk (Kitchen, 1985) and human milk (Hamosh *et al.*, 1985; Renner *et al.*, 1989). Their prevalence varies widely from one species to another. In many cases their presence may simply reflect the shedding of cell fragments into the milk, such as is amply demonstrated by the 'signets' or 'cytoplasmic crescents' associated with milk fat globules (section 12.7.5). Some such fragments may reflect cell damage, whereas others may be an inevitable accompaniment of secretory mechanisms. However, it is possible that some of the enzymes are actively secreted and benefit the young and/or the mother: lactoperoxidase, lysozyme and lipase may be of this sort.

Trace elements

Twenty two minerals are believed to be essential in the human diet. All have been detected in either cow's or human milks,

mostly in both (Flynn and Power, 1985). Certain minerals, e.g. calcium, phosphorus, sodium and potassium, have been discussed above, but the majority are 'trace elements', which are usually present in cow's and human milks at concentrations less than 1 mg/l (although that of zinc often exceeds 3 mg/l).

For most elements there is no simple relationship between maternal dietary intake and milk content. Trace elements may pass readily into milk, or be concentrated in, or largely excluded from, milk. They are rarely free in milk, but bound to a variety of ligands, a fact which has repercussions for their bioavailability in the neonate. For example, in cow's milk, most zinc and manganese is bound to casein, whereas copper is bound equally to casein and low molecular weight (<1000 Da) compounds. In human milk, binding patterns are quite different, e.g. most manganese and copper is bound to whey proteins, while zinc is bound to milk fat and low molecular weight compounds (Lonnerdal, 1985). For many elements, suitably sensitive methods have only become available in the last decade. This has led, for example, to a reduction of an order of magnitude in the accepted values for chromium and manganese in human milk (Casey *et al.*, 1985).

Vitamins

All the vitamins have been detected in milk, but, as for trace elements, only recently have sufficiently sensitive methods been available to permit reliable assays. Several analyses have been reported for cow's (see Cremin and Power, 1985) and human (see Jensen and Neville, 1985) milks. Certain vitamins are bound to specific proteins in milk (e.g. B_{12} and folate). Since vitamins are derived from the maternal diet, their milk concentrations vary accordingly. However, there is some evidence that vitamin C is synthesized in mammary cells in both cows and humans (see Cremin and Power, 1985).

12.4.6 INTERSPECIES DIFFERENCES IN MILK COMPOSITION

Several attempts have been made to rationalize the wide differences in milk composition evident in different species, e.g. Blaxter (1961), Davies *et al.* (1983), Oftedal (1984) and Jenness (1986). Essentially, two types of factor may be said to be involved: biological (i.e. higher level organizational), stemming from ecological, behavioural, developmental and metabolic differences; and physicochemical, relating to constraints imposed by factors such as solubility, electrical neutrality and osmotic equilibrium. Doubtless the two types of factor interact in complex ways.

Biological factors

A fruitful approach to the analysis of mammalian reproductive strategies as a whole is that introduced by McArthur and Wilson (1967), and developed by Pianka (1970), in which species are classed as exhibiting *K*- or *r*-selection strategies. *K*-selecting species produce few young, at an advanced developmental stage (precocial) – a strategy favouring stable environmental conditions. By contrast *r*-selection, for which there is close competition between species, produces large numbers of immature (altricial) young, so that the total population is kept close to the carrying capacity of the environment. Such criteria define extremes; many species exhibit intermediate characteristics. The classification has been shown to be applicable to milk composition. For example, *K*-species (e.g. primates, ungulates) secrete milk of lower protein content than *r*-species (e.g. rodents), a difference readily explicable by their different growth rates (R.D. Martin, 1984). The observation that the low protein content and prevalence of whey proteins, which characterize human milk, are associated with slow body growth and increased longevity led Bounous *et al.* (1988) to propose that human milk serves to extend the time available for the mental processes of learning.

In this accomplishment, by contrast with physical capacities, humans are supreme among animal species.

A consideration of clear importance is energy transfer, i.e. the capacity of the mother to furnish energy-yielding nutrients and that of the offspring to utilize energy in relation to their genetic potential. Several analyses (e.g. Oftedal, 1984; R.D. Martin, 1984) show that energy output in milk at peak lactation is correlated with maternal weight in a manner akin to Kleiber's law, i.e. $E_o = k\ W_m^{0.75}$ where E_o is energy output (kJ/day) and W_m is maternal mass (kg). Reproductive strategy was also found to be an important factor, since milk energy output in *r*-species was more than twice that of *K*-species of the same maternal body mass (Oftedal, 1984). A similar relationship exists for milk energy output and neonatal mass. The bioenergetics of lactation in marsupial and placental mammals have been discussed by Nicoll and Thompson (1987).

The habitats of some species would appear to exert overriding influence on milk composition because they encompass certain environmental extremes, e.g. those of Arctic and aquatic mammals. These species secrete milk with very high fat content, e.g. for polar bears 331 g/kg; and for grey seals 532 g/kg (Jenness, 1974). Such high concentrations may be related both to the rapid deposition of subcutaneous fat in the suckling young, which provides an insulating layer reducing heat loss, and to the high energy value of fat, which serves to maintain body temperature in conditions of external cold. An alternative explanation, which probably also applies to some desert species, is that high fat content represents a means of conserving water. But the low water intake of desert mammals may be surpassed by that of phocid seals, which abstain from eating and drinking during the several weeks of lactation, so that all water for milk secretion and other purposes must come from maternal body stores, chiefly in the form of fat (Bonner, 1984).

In one sense, milk composition is an abstraction; what is more crucial for the neonate is **intake** per day, a quantity which depends on suckling frequency, duration and volume consumed, as well as composition. Extreme variations in suckling frequency have been reported, e.g. at about 20 min intervals in mice, hourly in pigs, once daily in rabbits, once every 2 days in tree shrews, and once a week in the northern fur seal (Cross, 1977). Despite the importance of other factors, there appears to be an inverse relationship between suckling frequency and the concentration of milk constituents (R.D. Martin, 1984). Caution is, however, required in interpreting data from feral animals, both because the extent of supplementary feeding is unknown and because it is difficult to ensure that milk collection procedures have not altered the nature of the sample analysed.

Physiochemical factors

Whatever the biological pressures involved in shaping milk composition, ultimately physicochemical constraints must determine the limits of plasticity. It is an almost invariable observation that milk is isosmotic with blood plasma (Peaker, 1983) (milk of the elephant seal is an apparent exception; Peaker and Goode, 1978). This is advantageous in energy terms because secretion of either hyperosmotic or hypo-osmotic fluid would involve energy expenditure. Lactose is usually the major osmole in milk, though not in all species, the balance being due largely to the diffusible salts (Linzell and Peaker, 1971). However, lactose concentrations may vary quite widely, both during an individual lactation and between species (Cowie, 1969; Jenness, 1974; Chalk and Bailey, 1979). Therefore, lactose, or indeed any free sugar, has not been essential for the evolution of milk in general; and it seems possible that milk sugar evolved as a metabolically disposable alternative to salt, in species in which a

hot climate obliged the young to drink large amounts of milk for the sake of its water.

Although lactose is often the major determinant of milk water, under conditions where lactose secretion is reduced (e.g. during starvation) the relative proportions of diffusible salts in milk increase. In general, during established lactation there is an inverse relationship between the milk concentration of lactose and those of Na^+ and K^+ (Peaker, 1983). In contrast, under conditions when the paracellular route is operating (e.g. as a result of alveolar distension when milk is not removed) the milk concentrations of Na^+ and Cl^- increase and those of K^+ and lactose decrease, as equilibration with extracellular fluid (ECF) occurs: thus the normal inverse correlation between lactose and K^+ becomes positive (Peaker, 1983). Clearly, the relative concentrations of anions and cations in milk are governed by the requirement for electrical neutrality. In most eutherian species the milk concentrations of fat and protein depend largely on their rates of secretion relative to that of lactose and, hence, of water. Thus, in a study of many species, both fat and protein concentrations were shown to be negatively correlated with lactose concentration, whereas there was a positive correlation between fat and protein concentrations (R.D. Martin, 1984).

Analyses of milks of 37 species (Davies *et al.*, 1983) show a marked positive correlation between calcium and phosphorus concentrations, suggesting the prevalence of colloidal calcium phosphate. Moreover, total calcium and phosphorus contents in 33 milks were positively correlated with protein concentration, indicating an essentially constant stoichiometry for the calcium phosphate–casein complex (Jenness, 1979).

It is apparent that milk composition in different species is subject to multifactorial determination, with inputs arising from the environment, mother and offspring interacting in a complex and changing manner. The realization of these inputs in terms of milk composition is likely to be unique for each species, and constitutes a 'code' for neonatal development which bears comparison with the genetic code inherited at birth.

12.5 PROVISIONING THE MAMMARY GLANDS

12.5.1 GENERAL

It has been noted that the energy supply to the neonate in the form of milk often far exceeds that which is provided *in utero*: typically, parturition signals a marked and rapid increase in the provision of nutrients to the young as the route of transfer is switched from the placenta to the mammary glands. An important element of the maternal capacity to meet this new demand is the implementation of profound and concerted changes in whole-body metabolism. These changes may be categorized as: (i) enhancement of the entry rate (flux) of nutrients into body metabolic pools; (ii) diminution of nutrient utilization by tissues non-essential to lactation; (iii) augmentation of nutrient partitioning to the mammary glands and metabolically related organs, by means of cardiovascular changes; and (iv) stimulation of mammary substrate uptake by means of membrane-located carrier systems. The homeorhetic control of this repartitioning process is largely effected by the endocrine system (see Chapter 11), but other humoral and neural factors are also involved.

Most studies of the metabolic changes accompanying lactation have been performed on dairy species, for obvious commercial reasons. But often they are also suitable species from the experimental viewpoint, because of their large size, docile nature and ability to yield milk in response to abnormal milking stimuli (i.e. hand or machine milking). The unfortunate aspect of this bias is that ruminant metabolic activity exhibits distinctive features in consequence of the participation of rumen microbes in the digestion

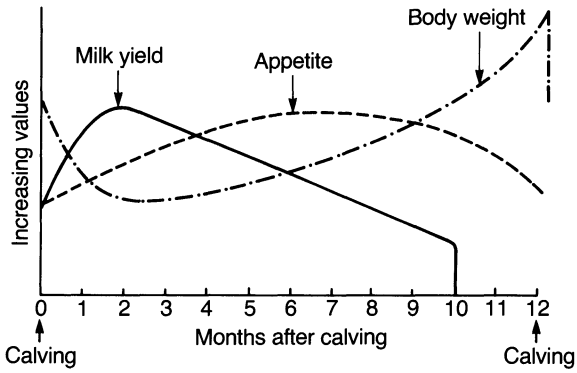


Figure 12.7 Changes in milk yield, body weight and appetite of dairy cows during lactation.

process, so that results from studies on dairy animals are not readily transposable to other species, for example non-ruminant herbivores, omnivores and carnivores.

One of the most notable features of body metabolism in dairy ruminants is the condition of negative energy balance, which in cows may persist for several weeks post partum. During this period the output of energy in milk exceeds that in food intake, and body weight is lost as tissue stores are mobilized (Figure 12.7).

Thus, studies on high-yielding cows showed that for the first 2 weeks of lactation 70% of milk fat was derived from adipose tissue stores, the mean figure for the first 12 weeks of lactation being about 40%. Such changes were achieved by reduced lipogenesis and increased lipolysis in adipocytes, together with increased lipid uptake by mammary cells (Bauman and Elliott, 1983; Vernon and Flint, 1984). Much less is known about repartitioning of protein metabolism at the onset of lactation, but muscle protein appears likely to serve a storage role analogous to adipose tissue. Bauman and Elliott (1983) estimated that in high-yielding cows muscle proteolysis might furnish 300 g/day of amino acids.

Ruminants differ most markedly from other species in respect of carbohydrate metabolism: because of rumen microcrobial activity, carbohydrates are largely fermented to the fatty acids acetate, butyrate and propionate, so that little glucose is absorbed into the blood from the gastrointestinal tract. Blood glucose concentrations in ruminants (largely derived from propionate by hepatic gluconeogenesis) are typically much lower than those in non-ruminants, whereas those of acetate are higher (e.g. see G.H. Smith *et al.*, 1983). Mammary glucose uptake is essential for milk secretion, so that lactation places additional demands on glucose supply. In goats, glucose flux at peak lactation is about double that in non-lactating animals (Annison and Linzell, 1964), a change which is due to a combination of reduced glucose uptake by adipocytes, reduced oxidation, increased gluconeogenesis and, probably, increased hepatic glycogenolysis and intestinal absorption (see Bauman and Elliott, 1983; Vernon and Flint, 1984). Calcium and phosphorus requirements in milk are met both by increased intake and absorption and by skeletal depletion (Bauman and Elliott, 1983).

Nutrient fluxes during lactation are thus markedly influenced by increased appetite. Hunger and satiety signals are generated in the CNS by pathways which, at the hypothalamic level, are believed to involve peptides of the cholecystokinin and opioid families (Baile and Della-Fera, 1988). Peripherally, signals may arise from physical (e.g. rumen distention) or chemical (e.g. ruminal concentrations of acetate and propionate) stimuli (Baile and Forbes, 1974). In dairy cows, in which food intake is the primary limitation on milk production, consumption during lactation may be four times maintenance requirements (Baile and Della-Fera, 1988).

Cardiovascular changes which result in an enhanced supply of substrates to the mammary glands are an important element of the partitioning of nutrients in lactating animals. Hanwell and Linzell (1972), working with rats, showed that not only was the onset of

lactation attended by a greatly increased cardiac output but there were also marked changes in its fractional distribution, such that mammary glands, liver and intestinal tract received larger proportions of the cardiac output, whereas skin received a smaller proportion. In some tissues, e.g. liver, both the flow rate per unit weight of tissue and the organ weight increased, whereas for others, e.g. skin, despite reductions in both weight and the fraction of cardiac output received, the increased cardiac output meant that flow rates per unit weight of tissue were maintained. Such comprehensive studies have not been carried out on dairy ruminants, but data reported for goats (A.J. Davis *et al.*, 1979) show a marked increase in mammary blood flow (MBF) in the periparturient period: MBF more than doubled between 7 and 9 days prepartum and 2 days post partum, with the largest component of the change occurring over the 2 days preceding parturition. During established lactation in cows and goats the mammary glands receive about 16% of the cardiac output (S.R. Davis and Collier, 1985).

The most extensive studies on MBF during established lactation were carried out on goats by Linzell and colleagues (see Linzell, 1974). The apparently labile nature of MBF, and its sensitivity to even minor stress, have led to an appreciable variation in reported values. Moreover, the several methods of measurement used do not give identical results (e.g. see Reynolds *et al.*, 1968). But for routine and repetitive MBF measurement in dairy ruminants Linzell's continuous thermodilution method (Linzell, 1966; Fleet and Mepham, 1983) has proved convenient and reliable when used in animals with surgically exteriorized mammary vein loops. More recently, blood flow probes utilizing the Doppler shift principle have been used to monitor mammary blood flow (MBF) (Fleet *et al.*, 1992). From extensive studies on goats it was concluded that, in full lactation, approximately 500 volumes of blood perfuse the mammary glands for each volume of milk secreted, a figure which also applies to cows (e.g. Peeters *et al.*, 1979). This ratio is, however, only an approximate index, and estimates of MBF derived from milk yield (MY) measurements are probably only accurate to within ± 30%. In late lactation, the MBF/MY ratio increases to over 1000 and, obviously, to infinity in dry animals.

The physiological factors which determine MBF *in vivo* remain a matter of uncertainty. There has been no shortage of reports of changes in MBF in response to various stimuli, e.g. adrenaline, vasopressin, 5-hydroxytryptamine, some prostaglandins and carbon dioxide are all mammary vasoconstrictors; while acetylcholine, histamine, adenosine and bradykinin are vasodilators (Linzell, 1974). The milking procedure and administration of exogenous oxytocin have also been reported to increase MBF in dairy animals, e.g. by 60% in cows (S.R. Davis and Collier, 1985). But definition of factors of physiological, rather than pharmacological, significance has been hampered by the possibility of atypical responses in anaesthetized animals in which MBF is depressed, and by anomalous results in isolated perfused gland experiments. For example, *in vivo* mammary ischaemia and bradykinin both induce reactive hyperaemia, whereas in perfused glands MBF is reduced (Linzell, 1974).

12.5.2 SUBSTRATE UPTAKE BY THE MAMMARY GLAND

The lactating ruminant mammary gland has proved to be a very useful organ system in which to study the quantitative relationships between substrate uptake and product output. The long-standing recognition of this utility is illustrated by the claim (Florkin and Stotz, 1977) that Cary's (1920) studies on lactating cows provided the first quantitative evidence for the importance of amino acids in protein synthesis in any biological system. In essence, this facility arises from a unique

combination of the attributes of (i) large gland size, (ii) favourable anatomical features (e.g. accessibility of the secretory product and of the gland's venous drainage), (iii) high secretory rate and (iv) absence of turnover of the secretory product in the duct system. This has meant that techniques such as determination of substrate arteriovenous (AV) concentration differences, isolated gland perfusion, and intra-arterial infusion can be performed much more easily for mammary glands than for other organs (e.g. see Fleet and Mepham, 1983; Mepham, 1987a).

The use of AV difference determinations combined with MBF measurements and techniques such as isotope dilution (see Fleet and Mepham, 1983; Mepham, 1993) has led to definition of the magnitude of substrate uptake by ruminant mammary glands in relation both to the output of milk constituents and to the metabolic economy of the whole animal. For example, it was shown by Annison and Linzell (1964) that in goats at peak lactation over 85% of the glucose flux was absorbed by the udder and that the glucose uptake largely accounted for lactose output, for a substantial proportion of glycerol of milk TG and, following oxidation, for about 40% of the total carbon dioxide produced by the glands. Whereas glucose is also the major precursor of lactose in non-ruminants (e.g. in pigs; see Linzell et al., 1969), the source of milk TGs exhibits much interspecies variability. In cows and goats, acetate, as well as being a major energy source for the mammary gland, is the principal precursor of those fatty acids of chain length up to C_{16} which are synthesized via the malonyl CoA pathway. By contrast, in certain non-ruminants glucose is the major source of these fatty acids, whereas non-ruminant herbivores utilize carbon from both acetate and glucose in mammary lipogenesis (section 12.4.3). A minor role is also played in ruminants by 3-hydroxybutyrate. However, the source of milk TG fatty acids of chain length C_{18} and above, and of a proportion of C_{16}, is quite different. In both ruminants and non-ruminant species these fatty acids are derived directly from blood, either from the plasma free fatty acid pool or from plasma lipoproteins following lipolysis within the lumina of mammary capillaries (section 12.4.3).

Historically, the blood precursors of milk proteins have been a matter of much uncertainty (see Mepham, 1986), and even now there appear to be grounds for some modification of views held widely since the 1950s. Isotopic tracer and AV difference studies in both whole animals and isolated organs suggested that plasma free amino acids were the principal, if not sole, precursors of milk-specific proteins, although in the case of non-essential amino acids there was evidence for intramammary synthesis, using nitrogen of certain amino acids (particularly that of arginine and ornithine) absorbed by the glands in excess of their output in milk (e.g. see Barry, 1961; Mepham, 1982). Subsequently, however, a minor contribution of amino acids from erythrocyte glutathione was suggested (Baumrucker, 1985), and quite recently it has been proposed that plasma oligopeptides might also contribute to the intracellular pool from which milk proteins are synthesized (Jois *et al.*, 1984). In so far as the latter proposal is based on AV determinations of amino acids in plasma samples before and after deproteinization, it depends on the assumption of complete deproteinization, which needs to be fully substantiated. A further dimension to the problem of identifying milk protein precursors is added by the claim that there is substantial intracellular degradation of protein prior to secretion (Mayer *et al.*, 1980; Oddy *et al.*, 1988). Despite the possible contribution of other sources, it remains likely that plasma free amino acids are usually the principal precursors of mammary-synthesized milk proteins: in some cases over 70% of an amino acid perfusing the gland is extracted from the blood plasma (see Mepham, 1982).

12.5.3 TRANSPORT OF SUBSTRATES INTO MAMMARY CELLS

The availability of substrates for milk synthesis depends not only on their rates of supply in blood perfusing the glands but also on their passage across at least two barriers, either or both of which might limit the transfer process, i.e. the capillary endothelium and the basolateral membrane of the secretory cell. For some substrates, additional barriers might be involved, namely erythrocyte membranes and intracellular membranes. Passage into interstitial fluid is governed both by capillary permeability and by the concentration gradient across the capillary wall, with molecules such as glucose largely diffusing through water-filled pores and lipid-soluble molecules also penetrating the endothelial membranes.

Transport of glucose across basolateral membranes of secretory cells has been studied almost exclusively in laboratory rodents, using non-metabolized analogues, such as 3-O-methyl glucose and 2-deoxyglucose, to circumvent problems associated with the rapid metabolism of glucose itself. Such studies suggest that glucose is transported into mammary cells by a system of the type which has been best characterized for mammalian erythrocytes, i.e. a process of facilitated diffusion. In rats, the activity of this putative transporter appears to be rate limiting for mammary glucose utilization (Threadgold *et al.*, 1982). The ability of the cells to absorb glucose is modulated by the animal's nutritional and hormonal status (Threadgold and Kuhn, 1984; Page and Kuhn, 1986), but the extent to which common mechanisms are involved in both is unresolved. Changes in glucose-transporting capability during mammary ontological development of murine cells, cultured *in vitro*, showed an 18-fold increase in V_{max} between mid-pregnant and mid-lactational developmental stages, suggesting an increase in numbers of transporters but not their affinity characteristics (Prosser and Topper, 1986).

Recently, Madon *et al.* (1990), using a wide range of techniques (such as photoaffinity labelling and polyclonal antibodies raised against previously characterized transporters), have proposed that the glucose transporter on the rat mammary cell plasma membrane is analogous to the rat brain glucose transporter, which has been designated 'GLUT 1'. This 55-kDa glycoprotein has been extensively studied, and competing models have been proposed to account for its mode of action (Silverman, 1991).

Threadgold and Kuhn (1979) reported that mammary cells cannot synthesize glucose because they lack glucose-6-phosphatase activity. Hence, glucose required for lactose synthesis (and other purposes) is derived from blood. Threadgold *et al.* (1982) suggested that cellular uptake was the rate-limiting step in overall glucose metabolism, and hence, because lactose is the major osmole of milk, of milk secretion as a whole. Certainly, when isolated perfused glands were deprived of glucose milk secretion was inhibited, whereas restoration of its supply re-established secretion (see Mepham, 1993).

Attention has only quite recently been turned to amino acid transport systems in mammary cells, and current progress is fairly limited. The notion that glutathione might participate in amino acid transport via the γ-glutamyl cycle (Baumrucker and Pocius, 1978) seems to have been invalidated by the discovery that γ-GTPase in mammary cells is oriented towards the extracellular surface, thus confining the role of glutathione to provision of glutamic acid, cysteine and glycine (Baumrucker, 1985).

The most promising approach to characterizing mammary amino acid transport systems appears to be that which seeks to extend the paradigm formulated by Christensen and colleagues (see, for example, Christensen, 1984), in which amino acids are divided into groups sharing affinities for distinct carrier

systems. For example, A, L and ASC systems have been described for neutral amino acids in many tissues and many species, as have separate anionic and cationic (e.g. Y^+) amino acid transporters. Systems are characterized by properties such as: amino acid specificity; sodium dependence or independence; and concentrative or exchange capability. The complexity of any description of amino acid transport is apparent when one considers the shared and/or overlapping affinities of different amino acids for specific transporters, interaction between different substrates such that transport might be either stimulated or depressed, and the potential modulation of transporter activity by factors such as turnover or endocrine influences (see Mepham, 1988).

Studies employing slices of bovine mammary tissue (Baumrucker, 1985) and diced murine mammary tissue (Neville *et al.*, 1980) suggested the presence in either or both of A, L, ASC and Y^+ carrier systems. However, a disadvantage of such preparations is that the normal spatial relations of the vascular, interstitial, intracellular and luminal compartments are disrupted, so that errors which might derive from extensive cell damage are compounded with others due to loss of basoapical orientation. Use of the isolated perfused guinea pig mammary gland preparation (S.R. Davis and Mepham, 1974) is thought to avoid such problems, and when used in conjunction with the 'paired tracer' technique of Yudilevich *et al.* (1979) permits measurement of both entry and efflux of amino acids. Evidence has been presented, using this technique, for the presence in guinea pig mammary glands at peak lactation of A, L, ASC and Y^+ systems, but A system activity was assessed as very low (Mepham *et al.*, 1984). Studies on transport in mammary tissue of glucose and amino acids are reviewed more extensively by Mepham (1988).

It was noted above that fatty acids of chain length C_{16}, C_{18} and longer are derived from serum lipoproteins. AV difference studies on cows and goats (see Annison, 1983) showed that TG from only the chylomicron and low-density (d=1.005–1.019) lipoprotein fractions are absorbed by mammary tissue. The uptake process is dependent on prior lipolysis of the TG, effected by lipoprotein lipase, an enzyme which is bound to the mammary capillary endothelium. The microscopical studies of Schoefl and French (1968), on tissue from laboratory rodents, showed that chylomicrons become embedded in mammary capillary endothelium, giving it a scalloped appearance. Earlier *in vitro* studies showed that lipoprotein lipase becomes strongly absorbed on to chylomicrons (D.S. Robinson *et al.*, 1955).

In lactating ruminants (but not in some laboratory rodents, see Hawke and Taylor, 1983) free fatty acids (FFAs) normally show no **net** AV differences across the mammary gland, but despite this their uptake is suggested by a fall in specific radioactivity when isotopically labelled FFAs perfuse the gland (see Annison, 1983). The apparent contradiction of these results is explained by postulating that FFA uptake is masked by simultaneous release into the mammary venous blood of fatty acids derived from TG lipolysis. Probably the most convincing evidence for lipolysis of TG in the mammary gland is the virtually identical pattern of incorporation of [^{3}H]glycerol and ^{14}C-labelled fatty acids into milk fat in experiments on goats in which isotopes were infused either as free [^{3}H]glycerol and [^{14}C]palmitate, or as [^{3}H]glycerol tri-[^{14}C]palmitate (C.E. West *et al.*, 1972). Lipolysis is not always complete and monoacylglycerols as well as FFA may be produced. The processes by which the latter traverse the capillary endothelium and basal membrane of the secretory cell are matters of uncertainty.

12.6 METABOLIC ACTIVITY OF MAMMARY CELLS

12.6.1 BIOSYNTHESIS OF MILK PROTEIN

Reference has been made above to the major classes of milk proteins. Before discussing their biosynthesis, recently reviewed in detail by Mepham *et al.* (1992), we need to consider briefly the impact which has been made by application of modern molecular biology and fractionation techniques and automated peptide sequencing methods.

Currently, there is emphasis on the application of DNA sequencing procedures to cDNA prepared from mRNA coding for the proteins and to isolated genes. With the aid of these techniques attention has also widened to include caseins and whey proteins of the cow (Brew *et al.*, 1970; Brew, 1972; Ribadeau-Dumas *et al.*, 1972; Grosclaude *et al.*, 1973; Brignon *et al.*, 1977), sheep (Jollès *et al.*, 1974; Richardson and Mercier, 1979), goat (MacGillivray *et al.*, 1979; Préaux *et al.*, 1979), deer (McDougall and Stewart, 1976), horse (Visser *et al.*, 1982), rabbit (Hopp and Woods, 1979; Dayal *et al.*, 1982), guinea pig (Hall *et al.*, 1984a, 1984b), rat (Brown *et al.*, 1977; Hirose *et al.*, 1981; Blackburn *et al.*, 1982; Hobbs and Rosen, 1982; Qasba and Safaya, 1984), mouse (Kinkade *et al.*, 1976; Hennighausen *et al.*, 1982a,b), human (Findlay and Brew, 1972; Metz-Boutigue *et al.*, 1984) and other species. References to β-lactoglobulin biosynthesis in various species may be found in Godovac-Zimmerman and Braunitzer (1987). By and large, all these milk proteins are relatively small and stable, and frequently exhibit variant forms. Unusual proteins which have also been found in certain milks include a variably phosphorylated 'whey acidic protein' in rats, mice and rabbits (R.M. Mckenzie and Larson, 1978; Hennighausen *et al.*, 1982a), a temperature-sensitive protein named 'galactothermin' in humans (Schade and Reinhart, 1970) and a distinctive whey protein appearing later in lactation in the tammar (Nicholas *et al.*, 1987).

The casein genes

The calcium-binding, micelle-forming properties of the caseins can now be related both to their molecular characteristics and to their organization within the genome. Several casein genes, or fragments of them, from rat, cow and guinea pig genomes, have been inserted into phages or plasmids, obtained as pure clones, and finally sequenced. Their construction resembles that of many other eukaryotic genes in having large non-coding nucleotide sequences (introns) intervening between stretches of coding sequence (exons) (Yu-Lee and Rosen, 1983; Jones *et al.*, 1985). From the analysis of these, and flanking, sequences it seems likely that an ancestral casein gene arose by the recruitment of four unrelated exons. These are a highly conserved non-coding 5′ exon of unknown function, an exon coding for the signal peptide that ensures secretion, a small exon coding for a phosphorylation site and an exon coding for hydrophobic amino acids. This evolutionary stage must have been followed by intragene duplication, especially to give multiple phosphorylation sites, and by intergene duplication to give the gene cluster that genetic analyses have recently identified (Matyukov and Urnyshev, 1980; Mercier and Gaye, 1983). Later on, separate mutations, deletions or insertions will have ensured that each gene acquired a characteristic individual sequence (Hobbs and Rosen, 1982; Rosen, 1987). Such an evolutionary scheme readily accounts for the three related calcium-binding bovine caseins (the highly phosphorylated α_{s1}- and α_{s2}-caseins, and the less phosphorylated β-casein) and for the homologous α-, γ- and β-caseins of rats and rabbits. It also accommodates the larger number of caseins identified, and located on chromosome 9, in the mouse (Gupta *et al.*, 1982) and the two caseins in humans. All these are of

the $\alpha_s$type. An unrelated gene is that coding for the κ-casein of bovine and ovine milk which, although it may carry a single phosphate residue, is differently constructed and does not bind calcium.

Expression of casein genes

Following the transcription of complex eukaryotic genes into correspondingly large RNA within the nucleus, the intron transcripts become excised. The remaining transcripts are simultaneously spliced together to form a mRNA with a continuous sequence of nucleotide codons corresponding to the protein to be translated; flanking sequences of the mRNA that do not become translated are believed to influence the stability and translatability of the rest. In the case of rat α- and β-casein genes, such splicing results in the linking of a single phosphorylatable sequence –Ser–Ser–Glu–, encoded by the 3′ end of exon IV, to a glutamate residue, encoded by the 5′ end of exon V. This creates the sequence –Ser–Ser–Glu–Glu – in which both serines satisfy the phosphorylation signal –Ser–X–glu– (X being any amino acid). In this way splicing converts a weak single phosphorylation site in a strong double one (Rosen, 1987).

The availability of cell-free translation systems, in which added mRNA can direct the synthesis of new, immunochemically detectable protein, and the availability also of [^{32}P]cDNA synthesized by reverse transcription of mRNA *in vitro*, has provided assays for mRNA species coding for individual casein or α-lactalbumin molecules (Rosen *et al.*, 1980). In tissue from intact rats, mice, rabbits and guinea pigs such mRNA is present throughout pregnancy and may even be detected in the virgin animal. Levels rise during pregnancy in the rat and mouse, and rise even more sharply with the onset of lactation (Nakhasi and Qasba, 1979; Hobbs *et al.*, 1982; Vonderhaar and Nakhasi, 1986). In the guinea pig α-lactalbumin mRNA clearly preceded casein mRNA in its appearance (Burditt *et al.*, 1981). Evidently, mRNA concentration is likely to be one important factor determining the rate of protein synthesis. This is supported by the finding that prolactin, cortisol and insulin, which jointly induce the greatest mammary differentiation and synthetic activity *in vivo* and *in vitro*, also maximally enhance tissue or tissue explant levels of casein- and α-lactalbumin mRNA. In the same way, progesterone, which inhibits the onset of milk protein synthesis *in vivo* and *in vitro*, also inhibits casein mRNA formation (Topper, 1970; Houdebine, 1976; Teyssot and Houdebine, 1981; Kulski *et al.*, 1983b). Prolactin, which probably plays the central role in this lactogenic complex of hormones, achieves its effect through a two- or three-pronged action. Its binding to receptors at the cell surface both enhances the rate of casein gene transcription and somehow stabilizes the resulting mRNA, whose turnover half-life within the cell increases from a few hours to several days (Guyette *et al.*, 1979; Teyssot and Houdebine, 1980; Kulski *et al.*, 1983a). In rabbits, prolactin has additionally been shown to stimulate the formation of ribosomal 28S RNA, although it confers no extra stabilization (Teyssot and Houdebine, 1980). This third prong of action may account for the increased numbers of ribosomes at lactogenesis.

The transcription of eukaryotic genes is promoted by the initial attachment of the large enzyme RNA polymerase immediately upstream (that is, on the 5′ side) of the transcription start codon ATG (A=adenine, T=thymine, G=guanine). The effectiveness of such a 'promoter' region in initiating transcription by the polymerase stems from its particular sequence of nucleotides. For many eukaryotic genes a TATA-like sequence is commonly found within promoter regions, and is believed to favour the binding of RNA polymerase. But other sequences may also influence transcription of the adjacent gene. Therefore, it may be presumed that the

strong expression of the major milk protein genes is likely to reflect a favourable constellation of nucleotides in their promoter regions. Comparative analysis of nucleotide sequences in the non-coding 5′ flanking region of three rat and bovine casein genes reveals them to be highly conserved, and to have an unusual TTTAAAT version of the TAT sequence (Yu-Lee *et al.*, 1986). At the same time, since the transcription of casein and α-lactalbumin genes is much accelerated by prolactin (or placental lactogen in late pregnancy) the promoters are likely also to be under the influence of an activating or inhibiting transcription factor controlled by this hormone. The details of prolactin action remain obscure, as do those of the positive influence on casein mRNA exerted by insulin.

Actions of steroid hormones

Cortisol and progesterone, respectively, reinforce and inhibit the transcriptional effects of prolactin on casein and α-lactalbumin genes, and have similar effects on the induction of lipogenic enzymes (Houdebine, 1976; Mayer, 1978; Rosen *et al.*, 1980; Teyssot and Houdebine, 1981; Kulski *et al.*, 1983a). Studies of the steroid hormone control of a variety of eukaryotic cells have recently focused upon short nucleotide sequences that bind steroid hormone–receptor complexes and enhance (or retard) the activity of nearby promoters. No single hormone is associated with any one unique sequence of nucleotides; rather, there appears to be a 'consensus' sequence that is able to bind a variety of steroid hormone–receptor complexes. The glucocorticoid-stimulated mouse mammary tumour virus, which has been much studied, has several such sequences upstream of the transcription start site as well as within the coding sequence itself. The 'enhancer' regions, or 'hormone response elements', appear to function at a distance (*trans* action) by enhancing the relevant promoter. A search for such sequences in the rat and bovine casein or α-lactalbumin genomes has revealed several, both in the 5′ flanking region and within the gene coding regions. They exhibit marked homology with glucocorticoid or progesterone receptor binding sequences associated with metallothionein, uteroglobin and lysozyme genes in other mammalian or avian tissues (Qasba and Safaya, 1984; Yu-Lee *et al.*, 1986). At the time of writing it seems possible that different steroid hormones may bind, through their receptors, to a common set of response elements, variously forming productive, partially productive or even inhibitory complexes (Ahe *et al.*, 1985). This situation appears to have arisen because steroid hormone receptors are all members of a multigene family, which also includes receptors for thyroid hormone and retinol (Chambon *et al.*, 1984; Gehring, 1987).

The scheme is attractive because one can readily envisage a mechanism by which two separate genes might evolve different sensitivities towards a common controlling hormone, for example the different sensitivities of α-lactalbumin and casein to cortisol (Nagamatsu and Oka, 1983). It also offers a plausible mechanism by which thyroid hormone can sensitize mouse mammary tissue to prolactin (Bhattacharjee and Vonderhaar, 1984).

Translation and processing

The time course of [^{3}H]leucine incorporation into protein has been followed in the rat mammary gland both *in vivo* and *in vitro* (Heald and Saacke, 1972). In both cases the protein product appeared initially in the ergastoplasm (rough endoplasmic reticulum), after about 30 min in the Golgi region, and from 30 to 60 min as an accumulation in the alveolar lumen. Such a pattern is explained by the initial translation of mRNA at the rough endoplasmic reticulum, and its passage through the stacked cisternae of the

Golgi apparatus, and its eventual concentration within the condensing vacuoles or elements of the *trans*-Golgi network. From these then arise the mature secretory vesicles which leave the cell by exocytosis at the apical membrane. Post-translational proteolysis, phosphorylation, glycosylation and complexing with calcium take place at specific points on this route (for review, see Mercier and Gaye, 1983).

The actual translation of mRNA at the ribosome has been little studied in the mammary gland, although the usual co-factors are required. The profile of aminoacyl tRNA precursors has been claimed to adjust, during lactation, to match the amino acid profile of milk protein (Garel, 1974). Therefore, little is known about possible controls at the translational step. However, in several reports the rates of synthesis of different milk proteins do not closely reflect the relative pool sizes of their mRNAs, so that some further element of control seems a distinct possibility (Burditt *et al.*, 1981; Geursen and Grigor, 1987). The translation of mRNA isolated from rat mammary gland or from polysomes of ovine udder, in a cell-free system from wheat germ, followed by the immunoprecipitation of individual milk proteins, was shown to reveal in each case an N-terminal 'signal' peptide extension. The amino acid sequences of several such radioactively labelled peptides have been determined by Edman degradation, and more recently from the sequence of the cloned cDNA (Lingappa *et al.*, 1978; Hobbs and Rosen, 1982; Mercier and Gaye, 1983). In ovine α_{s1}-, α_{s2}- and β-caseins these 15-residue sequences are highly conserved, with an N-terminal Met–Lys-$(X)_5$–Cys-$(X)_6$–ala– joining on to the rest of the molecule. Closely homologous to these are the signal sequences for rat α-, γ – and β-caseins and for mouse ε-casein. A positively charged residue separated by a stretch of hydrophobic residues from a connecting alanine is typical of many signal peptides in eukaryotic secretory proteins. The ovine κ-casein 21-residue peptide and the 18-residue β-lactoglobulin signal peptide are similar, though not homologous. However, the signal peptide of ovine α-lactalbumin lacks both a basic amino acid residue and a cysteine. Since the original discovery of a 'signal' peptide extension on the IgG light chain newly formed in a cell-free system (Milstein *et al.*, 1972), it has been established that possession of such a signal ensures the transport of newly synthesized proteins into the lumen of the endoplasmic reticulum. Several components of this transport machinery have been identified, but its actual mechanism and the reason for its wide tolerance of amino acid sequence remain unclear (Alberts *et al.*, 1983).

The translation of milk protein does not necessarily ensure its eventual secretion. Low rates of secretion, as in late pregnancy or during starvation, may partly reflect enhanced rates of degradation within the cell (Bienkowski, 1983; Hasan *et al.*, 1982). Caseins would appear to be good candidates for the class of proteins, rich in proline, glutamate, serine and threonine, claimed by the PEST hypothesis to be susceptible to rapid turnover (Rogers *et al.*, 1986).

Post-translational modifications

The immediate post-translational events in the formation and secretion of milk proteins must be inferred from studies of quite different secretory proteins such as those of the exo- and endocrine pancreas, salivary gland and even of viral coat proteins. Nevertheless, the secretions of other tissues, especially exocrine secretions, may be quite good models for the mammary gland. For example, phosphorylated salivary proteins have been characterized which, with their high proline content, appear not unlike casein (Wong and Bennick, 1980).

Therefore, guided by such studies, we can probably assume that a specific peptidase within the endoplasmic reticulum lumen cleaves the signal peptides of secreted milk

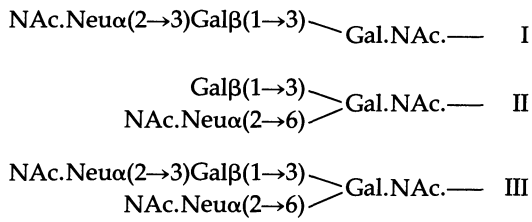
proteins. The remaining familiar milk proteins then pass through the stacked cisternae of the Golgi dictyosome to a still poorly defined *trans*-Golgi network of swollen tubules and condensing vacuoles, where the casein visibly assembles into micelles. The maturation of these vacuoles into more electron-dense secretory vesicles then precedes their actual secretion by exocytosis (Hollman, 1959). Secretion itself is described in section 12.7. In following this route out of the cell, some of the milk proteins acquire the phosphate or oligosaccharide residues that they are found to carry in milk. However, the extent of such post-translational modification is curiously variable, giving rise to the phenomenon of 'microheterogeneity' in the population of secreted molecules (Montreuil, 1980).

Studies of the phosphorylation of dephosphocasein by mammary preparations *in vitro* have shown the presence of an ATP-dependent, cyclic AMP-independent casein kinase embedded in the Golgi membrane and facing in towards the lumen. Phosphorylation requires Mg^{2+}, and is enhanced by Ca^{2+} and calmodulin (Bingham and Farrell, 1974; D.W. West and Clegg, 1983, 1984; Brooks and Landt, 1984). A casein kinase of guinea pig mammary Golgi membranes, which has been purified to homogeneity, appears to be tissue specific and phosphorylates only serine residues in the sequence –Ser–X–Glu–. Therefore, at least one further kinase must exist, to account for the phosphothreonine residues found in α_{s1}-casein variants (Pascall *et al.*, 1981; Moore *et al.*, 1985). In intact Golgi-derived vesicles casein phosphorylation is decreased by attractyloside or carboxyattractyloside, inhibitors of the mitochondrial adenine nucleotide transporter, and is enhanced by the nucleotide carrier alamethicin (D.W. West and Clegg, 1984). Direct transport of ATP into the lumen of Golgi membrane-derived vesicles from rat mammary gland has been described with possible exchange for 5'-AMP (Capasso *et al.*, 1989). Similar preparations also exhibit Mg^{2+}- or Ca^{2+}-dependent ADPase activity (Farrell *et al.*, 1992). These findings allow one to formulate a possible adenine nucleotide cycle in which cytosolic ATP entering the Golgi phosphorylates casein and generates ADP followed by AMP. The latter then passes back to the cytosol and mitochondria for rephosphorylation to ATP (Farrell *et al.*, 1992). This cycle has analogies with a uridine nucleotide cycle proposed to support lactose synthesis (see below), and emphasizes the essential cooperation of different subcellular compartments in the fashioning and secretion of milk products.

It may be noted, in parentheses, that the casein kinase activity frequently reported for other tissues, and even for the cytosol of mammary tissue, is not due to a true casein kinase, but rather to other protein kinases for which casein is merely a convenient substrate. The activity of mammary casein kinase (s) produces, in the cow, an α_{s1}-casein carrying typically eight phosphoserine residues, an α_{s2}-casein with up to 13 phosphoserines and a β-casein with usually five phosphoserines. Bovine κ-casein is sometimes referred to as non-phosphorylated, but in fact well exhibits the microheterogeneity referred to above by carrying one, two or three phosphate residues and varying amounts of carbohydrate per polypeptide chain (Vreeman *et al.*, 1986). Takeuchi *et al.* (1984) have proposed that phosphorylation and micelle formation of κ-casein precedes glycosylation, consistent with evidence for mainly surface glycosylation of the micelle (Slattery, 1978; Horisberger and Vonlanthen, 1980). Phosphorylation of the whey acidic protein appears not to have been studied. However, this is an unrelated protein of 14 kDa size and rich in cysteine residues which form disulphide links within the molecule.

The only commonly glycosylated milk proteins are κ-casein and lactoferrin, although the rat is unusual in that its α-lactalbumin is glycosylated (Brown *et al.*, 1977). Bovine κ-

casein carries three types of oligosaccharide in O-glycosidic linkage with serine or threonine:

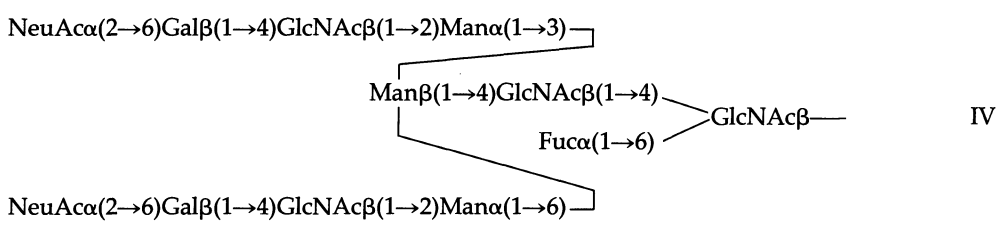


where I and II appear as uncompleted forms of the complete structure III (Van Halbeek *et al.*, 1980). The κ-casein of bovine colostrum carries more carbohydrate, in chains of greater complexity. The oligosaccharide chains of κ-casein from ovine colostrum also have the Gal-β(1→3)-Gal-NAc structure above, but carry Gal-β(1→4)-Glc-NAc-β(1→6) and Gal-β(1→3)-Gal-β(1→4)-Glc-NAc-β(1→6) attached to some of the Gal-NAc residues (Soulier *et al.*, 1980). The exact structure is not critical, but the presence of NAc-Neu residues (sialic acid residues) is. Such *O*-glycosyl chains are assembled by sequential glycosyl transfer from UDP-*N*-acetylgalactosamine, UDP-*N*-acetylglucosamine, UDP-galactose and CMP-*N*-acetylneuraminic acid in the Golgi apparatus. Since galactose may be attached in both 1→3 and 1→4 linkage, and *N*-acetyl-neuraminic acid in 2→3 and 2→6 linkage, it is evident that a family of glycosyltransferases, each with different sugar or positional specificity, must be employed. Since only the β14 galactosyltransferase and the α(2→3) and α(2→6) *N*-acetylneuraminyl (or sialyl) transferases have been studied in any detail in the mammary gland, their description will be deferred to the section on milk sugars, the synthesis of which they also catalyse.

In lactoferrin, by contrast, the oligosaccharide side chains are attached in *N*-glycosidic linkage with asparagine residues. Human lactoferrin has two glycosylation sites, from which five different oligosaccharide chains have been isolated. One of these has the structure shown in Equation IV with two others being variants of this (Spik *et al.*, 1982). This structure is characteristic of *N*-linked oligosaccharides, in which the Man_3-Glc-Nac_2 moiety is assembled in the endoplasmic reticulum and where the remaining *N*-acetylglucosamine, galactose, *N*-acetylneuraminic acid and fucose residues are attached in the Golgi apparatus (see Alberts *et al.*, 1983). As with the *O*-glycosyl chains, the particular structure formed reflects the family of glycosyltransferases, each with its particular specificity, that is present in the appropriate cell location.

12.6.2 ASSEMBLY OF THE CASEIN MICELLE

The major caseins of cow milk, α_{s1} and β, are strongly acidic and hence hydrophilic in their N-terminal regions, while being mainly neutral and hydrophobic in their C-terminal regions. The acidity arises particularly from the clustering of glutamate and serine phosphate residues. Under suitable ionic conditions these caseins assemble spontaneously into soap-like micelles (referred to henceforth as submicelles), the hydrophobic core of which is made up of the C-terminal regions of the polypeptide chains. The negatively charged outer regions ensure a strong mutual repulsion between nearby submicelles, which



therefore do not coalesce and flocculate unless excess of a strongly phosphate-binding cation such as Ca^{2+} is added. The less abundant α_{s2}-casein is not constructed in quite the same way. Serine phosphate groups are concentrated mainly, though not entirely, in the N-terminal moiety; the C-terminal region, while carrying a fair number of hydrophobic aromatic residues, also carries a distinct overall positive charge. Therefore, its arrangement within a submicelle is not yet fully evident. Like the α_{s1}- and β-caseins, κ-casein is highly amphopathic, but this time it is the N-terminal region that is very hydrophobic. It may be cleaved off by the action of the stomach enzyme chymosin (rennin) at a particular –Phe–Met– bond, forming the very insoluble 'para-K-casein'. The remaining moiety is the highly water-soluble 'caseinoglycopeptide', so called because it carries not only the phosphate residues but also the sialic acid-containing oligosaccharide chains.

Thus, it may be readily envisaged that κ-casein packs into the submicelle with its N-terminal region inward and its C-terminal region directed outwards, the branched oligosaccharide chains protruding into the surrounding medium. However, there is another particular feature of κ-casein, not shared by the α_{s1}- or β-caseins, that may be critical for the sharing out of κ-casein molecules among the different submicelles. This is the presence of two cysteine residues (three in the sheep), each of which forms a disulphide link with a cysteine of another κ-casein molecule, yielding a molecular population containing trimers, tetramers, pentamers, etc., up to greater than decamers (Talbot and Waugh, 1970). These disulphide links are probably due to the action of a sulphydryl oxidase in the endoplasmic reticulum, or else to a disulphide exchange enzyme employing oxidized glutathione made available by the sulphydryl oxidase (Kiermeier and Petz, 1967; Ferrier *et al.*, 1973; Janolino and Swaisgood, 1975).

The anticipated consequence of such linking is that each casein submicelle will acquire a larger or smaller cohort of κ-casein molecules confined, through their linking, to one sector of the spherical submicelle. Some submicelles will acquire no κ-caseins. Such mixed micelles, which form a family rather than a uniform population, may be those 15 to 20-nm-diameter particles that can be seen under the electron microscope, and that make up the subunits of the casein micelle proper, which is really a 'super-micelle'.

It is well established that the association of submicelles with each other requires millimolar concentrations of calcium and phosphate ions. From the histochemical localization of calcium this may occur mainly in the large vacuoles lying on the *trans* side of the Golgi stack, but might also include the peripheral regions of the *trans* cisternae (Wooding and Morgan, 1978). The growth of complete micelles is apparently self-limiting, particles not exceeding about 300 nm in diameter; this feature evidently underlies their remarkable stability, since particles of greater size would soon settle out. It is possible to devise a model according to which the size limitation arises from the construction of submicelles with differently sized cohorts of κ-casein. The accessibility of κ-casein in the micelle to lectins, to antibodies and to chymosin suggests its location predominantly in the outer shell of submicelles. Accepting the view that submicelles are held together by microcrystalline calcium phosphate bridges connecting the serine phosphates of the casein molecules, one can readily envisage a micelle assembly in which the central submicelles are poor in κ-casein and the outer ones are those in which abundance of κ-casein with its protruding oligosaccharide chains effectively prevents further submicelle accretion.

For further details on the ideas about casein micelle structure, the reader is referred to papers by Farrell (1973), Slattery and Evard (1973), Slattery (1978, 1979) and D.G. Schmidt (1980). According to Holt and Dalgleish (1986) the protrusion of κ-casein

from the surface even gives the micelle a hairy appearance. Whatever the structure that the casein micelle will eventually be found to possess, there is clearly a strong design feature that enables it to hold high concentrations of protein, calcium and phosphorus in a stable and mobile physical form in the milk. Equally clearly, this design stems from the detailed amino acid sequences of the proteins involved, and the structures of the genes from which they derive.

12.6.3 STRUCTURE OF LACTOFERRIN

The sequencing and recent X-ray crystallographic analysis of lactoferrin has related structure with function for another milk protein. The 80-kDa protein is folded into two lobes, reflecting a twofold internal sequence homology, each of which contains an iron binding site. The molecular structure is such that two tyrosine residues, one histidine and one aspartate are brought together from linearly distant parts of the polypeptide chain to create each, highly anionic and deeply buried, metal ion binding site. A carbonate ion is concomitantly bound to a nearby arginine residue, but there is no evident participation of the carbohydrate moiety of the protein (Baker *et al.*, 1987).

12.6.4 BIOSYNTHESIS OF MILK SUGARS

In addition to lactose and sialyllactose, which are prominent in rat and mouse milk, some milks contain lactose modified by the addition of other sugars to form trioses, tetraoses, pentaoses, etc. However, in this section we shall confine our attention to lactose and sialyllactose.

Rates of lactose formation

There are several ways in which the rate of mammary lactose (or sialyllactose) synthesis may be measured. In large domestic animals it can be calculated from the daily milk yield and the lactose concentration of milk. With the aid of a specially designed milking machine this has also been done for the guinea pig (Henderson *et al.*, 1983). In small animals, however, the milk yield is more usually gauged from the weight gain of the young (Brody and Nisbet, 1938) or from the increase in mammary tissue weight after a period of non-suckling (Hanwell and Linzell, 1972). A radiochemical method for determining rates of lactose synthesis *in vivo* employs the fact that plasma glucose is the sole precursor, at least in the rat, and measures the amount of [^{14}C]lactose formed over 30 min from plasma [^{14}C]glucose of known specific radioactivity (Carrick and Kuhn, 1978). These methods give similar results, but the shorter measurement time of the radiochemical method is more useful in following rapid changes in rate. Rates of lactose synthesis *in vitro* may sometimes reflect the enzymic capacity of the tissue quite well, but are wholly unreliable in reflecting its state of activation.

These several methods have delineated the quite different patterns of lactose synthesis during lactation in rats (Brody and Nisbet, 1938; Wilde and Kuhn, 1979), rabbits (Cowie, 1969), guinea pigs (Nelson *et al.*, 1951), goats (Fleet *et al.*, 1975) and cows (Rook and Campling, 1965). All species show a sharp rise in rate at, or before, parturition, but differ greatly in the time at which the rate peaks and later declines. Detailed studies of rats and mice have suggested that changes in tissue levels of lactose synthetase are responsible for these gross changes, and that enzymes earlier on the biosynthetic pathway are not limiting (Kuhn and Lowenstein, 1967; Kuhn, 1968; L. McKenzie *et al.*, 1971; Kuhn and White, 1977). In particular, the timing of lactose appearance around parturition is due to the withdrawal of progesterone (Kuhn, 1977), following which prolactin or placental lactogen stimulate the expression of the gene coding for the α-lactalbumin component of lactose synthetase (Turkington and Hill, 1968; Delouis, 1975, Nakhasi and Qasba, 1979). That this scheme holds true also for the

human is indicated by the timing of changes in progesterone and lactose (Kulski *et al.*, 1977) and by an extra leakage of α-lactalbumin into the bloodstream at lactogenesis (R.H. Martin *et al.*, 1980, 1981). The galactosyltransferase component of lactose synthetase is also induced at this time in the rat, albeit less dramatically, whereas its induction in the mouse seems to occur somewhat earlier (Palmiter, 1969; Turkington, 1971; L. McKenzie *et al.*, 1971).

In recent years these *in vivo* techniques have also revealed major short-term changes in rates of lactose synthesis according to nutritional status. In rats and mice withdrawal of food for 16 h completely shuts off lactose synthesis, which however restarts within 15 min of refeeding and climbs to 80% of the fed value by about 5 h (Bussmann *et al.*, 1984; Kuhn and Fisher, 1989). Partial restoration can also be achieved by the administration of glucose, maltose or sucrose into the stomach, but not by that of starch, fat or protein. In goats lactose synthesis also declines with starvation over 2 days, and is soon restored on refeeding (Faulkner *et al.*, 1981).

By contrast, lactose synthesis in the human breast is much less closely geared to maternal nutrition (Prentice *et al.*, 1986; Neville *et al.*, 1990), and this may be more typical of large animals with long breeding cycles and few young. The speedily reversible nature of the control of lactose synthesis in rodents that the above measurements imply is emphasized by findings with acini or slices of tissue *in vitro*. Surprisingly, the rates observed in preparations from 16 h-starved rats are indistinguishable from those in preparations from fed rats, the *in vivo* restraint having been lost during tissue preparation (Wilde and Kuhn, 1979; T. Page and N.J. Kuhn, unpublished work). Short-term control also manifests itself *in vivo* as a diurnal variation of lactose synthesis as well as of its sensitivity to nutritional change, both of which may in fact be consequences of diurnal variation in food intake (Carrick and Kuhn, 1978). In rats on a restricted food intake, the daily meal is rapidly followed by a burst of lactose synthesis that subsides to negligible rates during the rest of the day (Wilde and Kuhn, 1979).

Thus, the appearance of α-lactalbumin is probably mainly responsible for the initiation of lactose synthesis around parturition, and the tissue level of galactosyltransferase probably sets an upper limit to rates during established lactation. Actual rates during this period, however, are nutritionally controlled through short-term factors that may include insulin but really remain to be properly identified or established. Recent work has strengthened the long-suspected inhibition of milk formation by the accumulation of milk within the gland. More frequent milking of goats enhances production rates in a manner suggestive of the removal of some inhibitor (Henderson *et al.*, 1983). Isolated fractions of goat milk serum, of 10–30 kDa, inhibited both lactose and casein synthesis by rabbit mammary tissue *in vitro* or *in vivo* (Wilde *et al.*, 1987).

The lactose synthetase enzyme complex

The study of the purified enzyme complex 'lactose synthetase' is central to the study of just how lactose is formed and how this is controlled in the short-term. Early work with particulate fractions of mammary tissue from lactating guinea pigs, and then with partly purified enzyme from bovine milk, established the reaction:

$$\text{UDP-galactose} + \text{glucose} \rightarrow \text{lactose} + \text{UDP}$$

(Watkins and Hassid, 1962; Babad and Hassid, 1966). Later studies showed that the enzyme was specific for β-D-glucose, or for a very few analogues such as 5-thioglucose or 6-deoxyglucose (Kuhn *et al.*, 1980). UDP-galactose can only very poorly be replaced by UDP-4′-deoxyglucose or UDP-arabinose (Berliner and Robinson, 1982), and its utiliz-

ation is competitively inhibited by UDP or UDP-glucose. The effective irreversibility of the reaction can be ascribed to a large free energy change – unfortunately not yet determined – which at least in principle would allow the accumulation of high concentrations of lactose at its site of synthesis in the cell. In the course of purifying lactose synthetase from bovine milk, where it occurs in small but useful amounts, Ebner and his colleagues discovered two separate component proteins (Brodbeck and Ebner, 1966). One of these is the enzyme galactosyltransferase (EC 2.4.1.22), which by itself transfers galactose residues from UDP-galactose to non-reducing terminal *N*-acetylglucosamine residues of glycoproteins (Brew *et al.*, 1968). The product is a β1→4-galactoside that itself is often further modified by sialylation or fucosylation to form the finished glycoprotein. In cell-free preparations it is often convenient to use free *N*-acetylglucosamine as a galactosyl acceptor, but this is not strictly physiological. Galactosyltransferase occurs in many tissues, albeit at much lower concentrations than in the lactating mammary gland. The other protein turned out, unexpectedly, to be the long-known whey protein α-lactalbumin (Brodbeck *et al.*, 1967). The two proteins combine reversibly, in 1:1 stoichiometry, to create a 'lactose synthetase' that readily transfers galactose onto glucose. The enzymic activity remains the property of the transferase, which at the same time loses much of its ability to react with free *N*-acetylglucosamine. Combination with α-lactalbumin does not, however, impair galactosyl transfer onto glycoprotein acceptors, or indeed onto such analogues of *N*-acetylglucosamine as *N*-hexanoyl- and *N*-octanoylglucosamine. In the cell, therefore, it acts upon both glucose and glycoprotein substrates. In terms of reaction kinetics, the association with α-lactalbumin serves to lower the apparent Michaelis constant $K_{m\text{-glucose}}$ from about 1 M to 5 mM (Fitzgerald *et al.*, 1970; Klee and Klee, 1970), which begins to approach the physiological range (Page and Kuhn, 1987). This striking effect of α-lactalbumin has led to its designation as a 'specifier' or 'modifier' protein. In fact, it is better regarded as an allosteric ligand that, perhaps because of its unusually large size, exerts an unusually large effect.

α-Lactalbumin does not, in itself, participate in the mechanism of galactosyl transfer. The galactosyltransferases of different species readily interact with the α-lactalbumins of other species, even when these are phylogenetically distant enough not to cross-react with each other's antibodies (Ebner and Brodbeck, 1968). Great interest has been aroused by the finding of strong homology of α-lactalbumin with mammalian and avian egg-white lysozyme, with respect to both amino acid sequence and chain-folding structure (Brew, 1970; S.G. Smith *et al.*, 1987). The two proteins are believed to derive from a common ancestral precursor. Nevertheless, lysozyme cannot generally replace α-lactalbumin in lactose synthetase, nor does α-lactalbumin possess lysozyme activity.

For more detailed reviews on the enzymology and other aspects of lactose synthetase, readers are referred to Brew (1970), Ebner and Schanbacher (1974) and Brew and Hill (1975). Inositol is significantly, albeit slowly, galactosylated by lactose synthetase, giving the substance known as galactinol (White *et al.*, 1982). Both inositol and galactinol occur in small amounts in milk, but it is unclear whether they serve any function (Naccarato *et al.*, 1975). However, they are more significant constituents of semen, and it is of some interest that rat epididymis contains an α-lactalbumin-like protein that stimulates galactosyltransferase to use inositol as well as glucose (Qasba *et al.*, 1983).

Cation binding by lactose synthetase

Many glycosyltransferases are stimulated by millimolar concentrations of bivalent metal

cations such as Ca^{2+}, Co^{2+}, Mg^{2+} and Ni^{2+}, and especially by Mn^{2+} (see Navaratnam *et al.*, 1986). In the case of lactose synthetase, which has been investigated in greatest detail, an unexpectedly complex interaction with cations implicates both the galactosyltransferase and the α-lactalbumin components. α-Lactalbumin binds both Ca^{2+} and Zn^{2+}, one each at separate sites on the molecule (Murakami *et al.*, 1982; Murakami and Berliner, 1983). X-ray crystallography has defined the Ca^{2+} site as an 'elbow' of the polypeptide chain such that multiple coordination of oxygen atoms to Ca^{2+} lend it an association constant $K_{ass} = 10^{-6}$–10^{-8} M, depending upon the ionic strength of the medium (Mitani *et al.*, 1986; Stuart *et al.*, 1986). Binding of Zn^{2+}, also extremely tight, displaces the Ca^{2+}. It has been reported that the Ca^{2+} form of α-lactalbumin elicits a greater lactose synthetase activity than does the Ca^{2+}-free form (Musci and Berliner, 1985). However, lactose synthetase within permeabilized membrane vesicles, formed by the pinching off of Golgi membrane during the homogenization of mammary gland, does not show such a Ca^{2+} effect. At present, the physiological roles of Ca^{2+} and Zn^{2+} binding remain obscure. Although α-lactalbumin binds Ca^{2+} with the same avidity as does calmodulin or troponin C, its binding site is distinct and apparently not homologous.

By contrast with α-lactalbumin, the binding of metal ions by galactosyltransferase is clearly associated with activation. Kinetic studies of the purified enzyme have resolved two distinct types of Mn^{2+} binding site (Powell and Brew, 1976; O'Keefe *et al.*, 1980; Navaratnam *et al.*, 1988). Site 1 is half-saturated by about 10–30 μM Mn^{2+} in soluble forms of the enzyme, and is essential for activity. It may act as an electron sink in the drawing of electrons away from the bond to be broken, and towards the pyrophosphate grouping of UDP-galactose. Occupation of site 2 by Mn^{2+} gives a several fold further enhancement of activity (V_{max}) as well as affording thermal stability to the enzyme. However, *in vitro* this requires millimolar concentrations of Mn^{2+}, which are probably not physiological.

The question arises as to what are the natural activators of these two sites. Zn^{2+} and Co^{2+} ions can activate site 1, but do so less well than Mn^{2+}. Within intact vesicles arising from the Golgi membrane, permeabilized with the ionophore A23187, site 1 of lactose synthetase requires only 0.1–0.2 μM free Mn^{2+} for half-maximal activation. Yet in ruptured vesicles, or on solubilization, the enzyme requires 50–100 times as much. The interpretation of this remains uncertain (Kuhn *et al.*, 1991). At site 2 a quite different spectrum of cations can activate. Mn^{2+} can be replaced by Ca^{2+}, Mg^{2+}, Sr^{2+} or Ba^{2+}, and in fact Ca^{2+} has been envisaged as the natural occupant in view of its abundance in milk (Powell and Brew, 1976). However Ca^{2+} is not necessarily concentrated in the Golgi lumen, although Golgi-enriched vesicle fractions do accumulate it in an ATP-dependent marker (Neville and Watters, 1983).

The picture regarding site 2 is complicated by the finding that organic cations such as spermidine, lysyllysine or members of the series $^{+}NH_3$–$(CH_2)_n$–NH_3^{+} can also activate. Similar activation can be achieved with basic (cationic) proteins such as histone, clupeine, ribonuclease, melittin, etc. Mammary tissue and milk can be shown to contain basic proteins that activate at a concentration of only about 10 μg/ml, although these have not yet been characterized. Clupeine has been observed to enhance binding of glucose at the active site (Navaratnam *et al.*, 1986; Leong *et al.*, 1990).

Although these cation effects point to the presence of some regulatory system, it is too early to say how far they may mediate the short-term nutritional control of lactose synthesis described above. However, the activation of sialyltransferase, in the synthesis of

sialyllactose, by basic proteins implies that this form of control is not unique to lactose synthetase (Leong *et al.*, 1987, 1990).

Subcellular location of lactose synthetase

The Golgi location of lactose synthetase was first established by electron microscopy of mammary membrane fractions highly enriched in the enzyme (Keenan *et al.*, 1970). Subsequently, galactosyltransferase has been shown by immunofluorescence techniques to lie in the *trans*-most two or three cisternae of the Golgi stack, in HeLa cells and in hepatoma cells (Roth and Berger, 1982; Slot and Geuze, 1983). The enzyme is anchored, at least partly in dimeric form, in the Golgi membrane, with the active site directed towards the lumen (Brew, 1970; Kuhn and White, 1975a). Detergent is required to solubilize it, at which point it becomes extremely labile unless stabilized with detergent, phospholipid, Mn^{2+}, site 2 activators or serum albumin.

The properties of the enzyme have recently been reviewed (Ram and Munjal, 1986). The significance of this Golgi cisternal compartmentation lies in the fact that secretory (glyco)proteins, and (glyco)proteins destined for the cell membrane, pass through the Golgi lumen and acquire many of their glycosyl residues there. Indeed, self-glycosylation results in galactosyltransferase also being a glycoprotein (Trayer and Hill, 1971). α-Lactalbumin, which in the rat is also glycosylated (Brown *et al.*, 1977), is presumed also to pass through the lumina of successive Golgi cisternae, interacting reversibly with the galactosyltransferase and thus creating the lactose synthetase complex.

The important question now concerns the nature and concentrations of organic cations and bivalent metal ions in this Golgi compartment. Present evidence suggests that at low concentrations Mn^{2+} does not readily cross the Golgi membrane. Histochemical studies tend to locate high concentrations of free Ca^{2+} in the vacuoles distal to the *trans*-cisternae, although possibly including the outer rims of the latter (Wooding and Morgan, 1978). The high Ca^{2+} and Mn^{2+} affinities of α-lactalbumin and galactosyltransferase, on the other hand, seem to imply luminal concentrations of such ions in the range 10^{-6}–10^{-7} M.

Substrate access to the Golgi lumen

Enzyme compartmentation raises questions of substrate access. The access of cystolic glucose and UDP-galactose to the Golgi lumen has been studied in pinched-off membrane vesicles that retain internal α-lactalbumin. Both substrates readily penetrate the membrane to reach the lactose synthetase, but the product lactose does not diffuse out again. It can be released, however, when the vesicles are osmotically ruptured following exposure to high concentrations of penetrating solutes. With this as a monitoring device, one can show that the membrane specifically associated with lactose synthesis admits not only glucose but a wide range of sugars or sugar alcohols. Low temperatures are required to study this rapid penetration of polyols, which include D- and L-sugars and inositol, but not polyols of molecular weight greater than 200–300 Da (White *et al.*, 1980, 1981). Such low structural specificity, lack of saturability, and also absence of competitive inhibition, are consistent with the properties of a water-filled pore of about 1 nm diameter (White *et al.*, 1984). The passage of inositol is of interest, in view of its galactosylation and secretion into milk.

The probable equilibration of cytosolic and Golgi lumen glucose pools, together with the Golgi origin of the aqueous phase of milk, has given rise to the possibility that milk glucose concentrations may afford a direct guide to intracellular glucose concentrations (Kuhn and White, 1975b). This is of interest in so far as the latter might be a regulatory feature of lactose synthesis. A number of

studies following up this suggestion have supported the equivalence between milk glucose and intracellular glucose (see Faulkner and Peaker, 1987). However, whereas rates of lactose synthesis appear to correlate well with milk glucose concentrations in some cases, e.g. goats (Faulkner and Peaker, 1987) and humans (Neville *et al.*, 1986, 1990), they do not correlate in other cases, e.g. rat (Page and Kuhn, 1987; Grigor *et al.*, 1989). Nevertheless, there remains the interesting possibility that, with care in the interpretation, the minor components of milk may form a useful and readily accessible index of intracellular substances in the mammary gland.

The few studies carried out on the mechanism of UDP-galactose penetration of the Golgi membrane from mammary tissue have shown a specificity that includes UDP-galactose and UMP, but excludes UDP-glucose, UDP-glucuronate and UDP. The significance of this, which clearly implies a carrier-mediated transport, lies in the presence of a nucleoside diphosphatase (thiamine pyrophosphatase) in the Golgi apparatus. Through the action of this enzyme the UDP originating from the lactose synthetase reaction becomes hydrolysed to UMP, which is then transported back into the cytosol. There it is converted via UDP, UTP and UDP-glucose back to UDP-galactose which re-enters the Golgi lumen. Figure 12.8 depicts the way in which this organized system of coupled enzymes and transporters is envisaged within the Golgi cisterna, linked with the supporting reactions of the cytosol.

There is, in effect, a cycle of uridine nucleo-

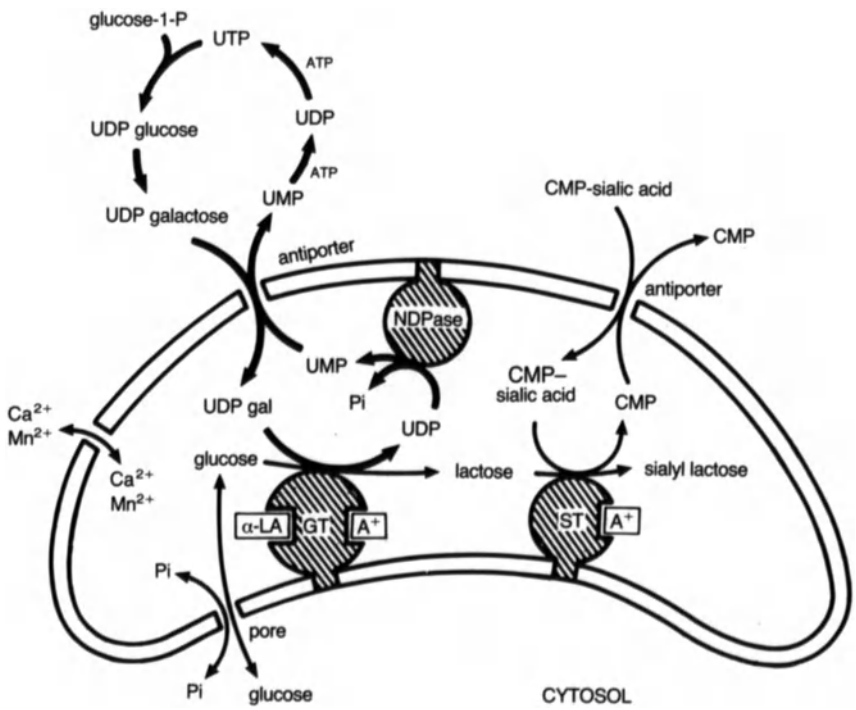


Figure 12.8 Current view of a *trans*-Golgi cisterna, showing coupled enzyme systems and membrane transporters, linking Golgi luminal and cytosolic processes. GT, galactosyltransferase; ST, sialyltransferase; NDPase, nucleoside diphosphatase; α-LA, α-lactalbumin; A^+, cationic activator. The uridine nucleotide cycle is shown with bold arrows.

tide molecules carrying galactose residues into the Golgi lumen on their backs (Kuhn and White, 1977).

The role of nucleoside diphosphatase, long regarded as a histochemical marker for Golgi apparatus in secretory tissues, is quite interesting. Its location, together with the membrane impermeability towards UDP, ensures the destruction of luminal UDP, which would otherwise powerfully inhibit lactose synthetase as a competitive analogue of UDP-galactose, while preserving cytosolic UDP. Hydrolysis of UDP costs the cell an extra 'high-energy' bond per lactose synthesized. Subsequent experiments with liver Golgi membranes have confirmed this scheme (Brandan and Fleischer, 1982; Barthelson and Roth, 1985) and have extended it to the transport of other sugar nucleotides as well as the sulphate donor 3'-phosphoadenosyl-5'-phosphosulphate (Perez and Hirschberg, 1986; Hirschberg and Snider, 1987). By analogy, it appears that a single carrier in the membrane may, as a symporter, mediate both the entry of UDP-galactose and the exit of UMP. This might justify regarding the nucleoside diphosphatase as a sort of pump that supplies a gradient of UMP, which drives the uptake of UDP-galactose. Possibly this is the chief justification for the hydrolysis of UDP, rather than the relief from inhibition of lactose synthetase.

The origins of other milk sugars

The oligosaccharides that appear in the milk of humans, rats and mice, and in the colostrum of cows and sheep, form a fascinating family. Even more so are those characterized in such abundance in the milk of marsupials such as the tammar wallaby (*Macropus eugenii*) and grey kangaroo (*Macropus giganteus*), in which galactosyl-β(1→3) galactosyl-β(1→4) glucose is prominent (Messer *et al.*, 1980), and in the milk of monotremes such as echidna (*Tachyglossus aculeatus*) or platypus (*Ornithorhynchus anatinus*), in which sialyl- and fucosyllactoses are particularly found (Messer, 1974; Kamerling *et al.*, 1982; Messer *et al.*, 1983), as well as a wide variety of oligosaccharides (Amano *et al.*, 1985). However, only a little enzymology has been done. These trisaccharides, and the higher oligosaccharides that also occur, are probably all formed by extension of lactose.

An α(2→6)sialyltransferase has been purified from bovine colostrum in both 56-kDa and 43-kDa sizes (Paulson *et al.*, 1977). The latter may be a degraded form of the former. However, the best sialyl acceptors for this enzyme are β-galactosides of *N*-acetylglucosamine or its derivatives, so that its function probably lies rather in the synthesis of glycoproteins.

Rat mammary tissue contains particulate sialyltransferase activity, which exhibits a K_m towards lactose of about 1 mM and yields mainly α(2→3)sialyllactose with a little α(2→6)sialyllactose (Carlson *et al.*, 1973; Leong, 1988; Leong *et al.*, 1990). A detailed study has been made of the way in which this enzyme, acting within tightly sealed vesicles of Golgi membrane origin, can be fed with lactose by lactose synthetase (Leong *et al.*, 1990). The two enzymes constitute a coupled enzyme system within the same Golgi compartment, in which free lactose liberated by the one becomes the substrate for the other. This is depicted in Figure 12.8.

The K_m value given above is of interest, as it may indicate the likely concentration of lactose within the normal Golgi cisternal lumen. Were it correct, it would imply that the concentration of lactose to milk values occurs only subsequent to the Golgi stack, possibly in the condensing or secretory vesicles.

An interesting feature of the detergent-solubilized sialyltransferase is its marked activation by the basic proteins histone and clupeine (Leong, 1988; Leong *et al.*, 1990). This is reminiscent of the behaviour of galactosyltransferase, and may point to a common regulating mechanism.

12.6.5 BIOSYNTHESIS OF MILK FAT

No biochemical aspect of mammary function has attracted so much attention over the last 30 years as the problem of milk fat synthesis. The complexities arise from the variety of the constituent fatty acids, their different origins within the body and our ignorance of the intracellular signalling mechanisms that mediate hormonal control of their synthesis and esterification. Yet much of the metabolic activity of the gland is directed towards making this energy-rich milk solid, so that the process has a disproportionate influence upon mammary activity as a whole. Reviews on this subject include those by S. Smith and Abraham (1975), Patton and Jensen (1976), Mayer (1978), Dils (1983), Munday and Hardie (1987) and Clegg (1988).

There is an enormous variety of fatty acid types encountered in milk fat, and the finding that, although the milk of a given species may have a 'characteristic' fatty acid profile, nevertheless it can be markedly influenced by the amount and the nature of fat in the diet. We owe this detailed knowledge to the exploitation of the high resolving power of gas–liquid chromatography, which has been extended even to the separation of intact triacylglycerols according to their carbon number, and hence their fatty acid complement. As far as the origins of milk fat are concerned, an important achievement of early workers in the field was to establish that the complex lipids of plasma are not taken up directly into the mammary gland. Rather, they are hydrolysed extracellularly, and the subsequently absorbed fatty acids and glycerol are then assembled anew into complex lipids within the cell (Kinsella, 1968; Easter *et al.*, 1971). It is now also clear that the short- and medium-chain fatty acids, and some of the $C_{16:0}$ fatty acids, are synthesized *de novo* within the secretory epithelial cells of the mammary gland. The remaining fatty acids are drawn from the triacylglycerol of plasma lipoproteins, and it is through their provision that the influence of dietary lipids is exerted. So far as is known, all these fatty acids enter a common pool of fatty acyl coenzyme A (CoA) within the cell, and the esterifying enzymes draw upon this pool with an element of specificity for their chain length or degree of unsaturation.

Esterification of fatty acids

Esterification is the only significant fate of long- and medium-chain fatty acids in the mammary gland, as oxidation appears to be a very minor process. The great majority of the fatty acids are assembled by enzymes of the rough endoplasmic reticulum to form the triacylglycerol of the milk fat globule (Stein and Stein, 1967). However, a not insignificant proportion go to replenish the phospholipids – particularly phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in the cow – that are lost from the apical membrane when this gives rise to the milk fat globule membrane in the course of secretion (Patton and Jensen, 1976). It is the task of the enzymologist not only to document the metabolic pathways by which these complex lipids are formed, but also to account for the preferential attachment of particular fatty acids at particular positions of the glycerol moiety. The insoluble or the detergent-like nature of all these enzyme substrates has greatly hampered the application of traditional enzyme kinetics. Indeed, there is good evidence that long-chain fatty acids and their coenzyme A derivatives are largely attached to particular binding proteins (Keuper *et al.*, 1985; Morgensen *et al.*, 1987), so that the concentrations of their 'free' forms are probably around micromolar values.

sn-Glycerol 3-phosphate is the primary acyl acceptor for fatty acids, being formed partly by the NADH-dependent reduction of dihydroxyacetone phosphate that arises from glycolysis, and partly from the ATP-dependent phosphorylation of free glycerol that arises from the hydrolysis of the plasma triacylgly-

cerol supplying the fatty acids (see S. Cooper and Grigor, 1980; Dils, 1983). It is tempting to draw parallels with intestinal mucosa, where the dietary triacylglycerol is similarly disassembled, absorbed and reassembled. But, unlike in that situation, 2-monoacylglycerol is unlikely to be an important acyl acceptor in the mammary gland.

The phosphatidate formed by two successive acyl group transfers can then react with cytidine diphosphate choline (or cytidine diphosphate ethanolamine) to form phosphatidylcholine (or phosphatidylethanolamine). These are transported to the membranes of the cell by a mechanism that is not well understood. Alternatively, the phosphatidate undergoes enzymic hydrolysis to *sn*-1,2-diacylglycerol, the further esterification of which yields the triacylglycerol of milk. The non-random positioning of different fatty acids is intriguing (Breach *et al.*, 1973). Short- and medium-chain ones are found exclusively on the 3-hydroxyl, which typically also carries unusually long or unsaturated fatty acids.

In the milk fat of ruminants, other than sheep and goats, and of man and whales, palmitate occurs particularly at the 2-hydroxyl. This circumstance does not arise in carcass fat (except pig lard), in which the 2-position usually acquires unsaturated fatty acids. Although the fatty acid specificities of the three enzymes that acylate these hydroxyl groups have not been exhaustively examined, there are signs that the complement of fatty acids is mainly determined by the specificities of the two enzymes acylating positions 1 and 2 of glycerol phosphate (Breach and Dils, 1975; Kinsella, 1976; Marshall and Knudsen, 1977, 1979; S. Cooper and Grigor, 1980). The concentration of shorter chain or of unusual fatty acids at position 3 appears to owe more to fatty acid exclusion from positions 1 and 2 than to their positive selection by the diacylglycerol-specific acyltransferase (Marshall and Knudsen, 1979).

A feature of triacylglycerol synthesis that remains poorly researched in mammalian tissues is the manner in which the product is directed to that compartment of the cell where it is either stored (e.g. the cytosol in adipose tissue) or from where it is secreted (the Golgi apparatus in liver and intestine; the cytosol in mammary tissue). A good autoradiography study of newly formed [^{14}C]triacylglycerol, in cells of lactating-mouse mammary gland, clearly specified the rough endoplasmic reticulum as the site of synthesis (Stein and Stein, 1967). In another electron microscopic study, not actually in mammary gland, the fat droplet was ingeniously envisaged as growing within the lumen of the endoplasmic reticulum, gradually forcing apart the opposite membranes, which eventually come to form barely detectable strands overlying the surface of the fat droplet that now lies, essentially, within the cytosol (for references see Zaczek and Keenan, 1990).

Plasma origin of fatty acids

Milk fatty acids originating from the plasma are drawn from the chylomicrons and very low-density lipoproteins (VLDLs) that perfuse the mammary gland (Barry *et al.*, 1963; Schoefl and French, 1968; Bishop *et al.*, 1969; C.E. West *et al.*, 1972), and whose triacylglycerol is hydrolysed to monoacylglycerol and free fatty acid by lipoprotein lipase (Scow *et al.*, 1972). Lactating mammary tissue is immensely well endowed with this unusual enzyme, whose prolactin-dependent induction at lactogenesis is a major factor in the redirection of fatty acids (nutrient partitioning) away from adipose tissue and to the mammary gland (McBride and Korn, 1963; D.S. Robinson, 1963; Zinder *et al.*, 1974; Mendelson *et al.*, 1977). Extrapolating from studies on adipose tissue, we may presume that mammary lipoprotein lipase is synthesized within the epithelial cells and then

transported to an extracellular site on the luminal walls of the blood capillaries, where it binds and 'digests' passing chylomicrons and VLDL (see electron micrographs by Schoefl and French, 1968). After further hydrolysis of the immediate product monoacylglycerol, at an extracellular site not fully identified, all the fatty acids are absorbed into the cells, activated to their CoA thioesters, and re-esterified into milk fat. There is some evidence that monoacylglycerol may partly be absorbed and directly esterified in the mammary gland of pigs (Bickerstaffe and Annison, 1971a), but this seems not to be the usual pathway (Hansen *et al.*, 1986).

In general it seems that the fatty acids taken up from the plasma are utilized unchanged for milk fat synthesis. However, in the mammary glands of the cow, goat and sow a stearoyl CoA desaturase has been identified; the enzyme is located in the microsome fraction of tissue homogenates, and employs NADH (and presumably oxygen) to convert stearoyl CoA into oleoyl CoA. This ensures that the milk fat is not overendowed with saturated fatty acid arising by the biohydrogenation of unsaturated dietary fatty acids in the rumen (Bickerstaffe and Annison, 1971b; Kinsella, 1972; Davies *et al.*, 1983).

Biosynthetic origin of fatty acids within the mammary gland

The *de novo* synthesis of fatty acids is essentially a reductive condensation of acetyl and malonyl residues provided as their 'activated' thioesters. So great is the contribution of this process to mammary metabolism that it causes the respiratory quotient (ratio of carbon dioxide formed per oxygen molecule consumed) of the tissue to rise as high as 1.6. An early study with rat mammary slices nicely documented the shift of respiratory quotient, from below 1.0 to about 1.6, that accompanies the transition from pregnancy to full lactation (Folley and French, 1949). The formation of short- or medium-chain fatty acids is one of several unique metabolic features displayed by the mammary tissue of many species. Biosynthetic studies have well documented the retention of this feature by slices or explants of rat, rabbit, mouse and goat mammary gland incubated with [^{14}C]glucose or with [^{14}C]acetate *in vitro* (Wang *et al.*, 1971; Strong and Dils, 1972; S. Smith and Abraham, 1975; Grunnet and Knudsen, 1979). Therefore, it came as a surprise when the fatty acid synthetase, which was eventually purified, proved to generate mainly long-chain fatty acids and even to be indistinguishable from the synthetase of liver. In fact, the chain length of the product is markedly influenced by the ratio of the acetyl CoA and malonyl CoA substrates presented to the enzyme, a high ratio favouring the starting of new chains at the expense of finishing the old. Growing acyl chains, covalently attached to the 4-phosphopantetheine prosthetic group of the synthetase, are finally released by hydrolysis of the thioester by a thioesterase component (thioesterase I) of the enzyme complex.

A novel finding in rat and rabbit mammary gland, as opposed to non-mammary tissues, was a second, soluble, thioesterase of markedly shorter chain-length specificity. The action of this thioesterase II forestalls that of thioesterase I, so that a shorter-chain fatty acid is the product in the intact cell (Knudsen, 1979; Dils, 1983). The thioesterase II of rat mammary gland has been well characterized with respect to its amino acid sequence (Naggart *et al.*, 1987) and its induction during pregnancy (S. Smith and Ryan, 1979). It is interesting to note that the two non-protein milk substances that are unique to milk, namely medium-chain fatty acids and lactose, both require unique proteins for their synthesis, namely thioesterase II and α-lactalbumin.

However, the termination of fatty acid chains is also favoured by the presence of suitable fatty acid acceptors, although the

identity of those that operate within the mammary cell is not entirely clear. Nevertheless, these fatty acids, together with those arising from the plasma, are activated to their coenzyme A thioesters by an ATP-dependent fatty acyl CoA synthetase. In ruminant udder, where a weak medium-chain thioesterase II also appears to exist, the dominant process is a direct acyl transfer from the synthetase to coenzyme A, with conservation of the high-energy thioester bond (Grunnet and Knudsen, 1983; Mikkelsen *et al.*, 1985). This, too, is enhanced by the presence of suitable acyl CoA acceptors, possibly accounted for by a specific acyl CoA-binding protein such as has been purified from liver (Morgensen *et al.*, 1987)

Precursors for fatty acid synthesis

Complexity also surrounds the source of acetyl CoA and malonyl CoA precursors for fatty acid synthesis, and the origin of NADPH used as reducing agent. In most non-ruminants the acetyl CoA arises in the cytosol of the mammary epithelial cell from the action of ATP-citrate lyase upon citrate; this in turn originates from the mitochondria following its formation from pyruvate, which itself arises from carbohydrate, alanine or lactate. In ruminants or non-ruminant herbivores, however, the deficiency of ATP-citrate lyase spares the use of carbohydrate, and acetyl CoA arises instead from free acetate taken up from the plasma (S. Smith and Abraham, 1975). When the properties of mammary fatty acid synthetases are studied, it is found that butyryl CoA is as acceptable as acetyl CoA in initiating growth of the fatty acyl chain (Nandedkar and Kumar, 1969; Lin and Kumar, 1971). It is therefore of interest that ruminant mammary tissue is able to utilize acetoacetate and 3-hydroxybutyrate, which reach the plasma from the rumen, and to reduce these to butyryl CoA (Palmquist, 1969). These two ketone bodies can therefore serve as precursors of both the butyryl residues and the terminal four carbon atoms of long-chain residues of milk fat. In these ways it is seen that fatty acids can be fashioned out of a wide variety of precursors drawn from the plasma, and it is evident that the relative importance of each varies according to species and to nutritional state.

Reducing equivalents, in the form of NADPH, are generally believed to originate largely from the pentose phosphate cycle. Indeed, it was partly through the investigation of NADPH generation in the mammary gland of the lactating rat that the cycle and its role in lipogenesis generally was recognized (Abraham *et al.*, 1954; Glock and McLean, 1954). Mammary tissue is especially rich in the coenzyme NADP(H) and in the oxidative and non-oxidative enzymes of the cycle (McLean, 1958a,b; Gumaa *et al.*, 1971). In mammary preparations *in vitro*, the addition of glucose greatly enhances the incorporation of [^{14}C]acetate into fatty acid (Hirsch *et al.*, 1954). This is believed to reflect the provision of NADPH by the first two enzymes of the cycle, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Consistent with their proposed role is their marked induction during the onset and course of lactation (McLean, 1958a). However, some NADPH almost certainly arises from the oxidation of malate in the rat. Interestingly, the formation of NADPH in sheep udder does not inevitably require the presence of glucose and the operation of the pentose phosphate cycle. Greenbaum and his colleagues have shown that acetate itself can be the source of reducing power, and have proposed a plausible metabolic pathway by which NADPH is ultimately generated through the action of a cytosolic isocitrate dehydrogenase, which is quite active in this tissue (Gumaa *et al.*, 1973). The challenge of estimating just what proportion of NADPH arises by way of the pentose phosphate cycle has been met, both *in vivo* and *in vitro*, by experiments in which the fates of differently ^{14}C-labelled glucose are monitored; the in-

terpretation is complex (Wood *et al.*, 1965; Katz and Wals, 1972; Chaiyabutr *et al.*, 1980).

This is not the place to describe the fascinating structure of the very high molecular weight fatty acid synthetase that enables it to be loaded with acetyl (or butyryl) and malonyl residues, to condense them reductively into saturated fatty acids, and finally to transfer the latter onto water or coenzyme A. Suffice it to remark that this 'polyfunctional protein' appears to have evolved from separate component enzymes by a process of fusion at the gene level (Hardie *et al.*, 1986). Important though these reactions may be in chemical terms, it is the preceding enzyme acetyl CoA carboxylase that catalyses the major rate-limiting and hormonally regulated step of lipogenesis. This enzyme is subject to both long-term inductive control (as, indeed, is also the fatty acid synthetase), and to short-term allosteric and phosphorylative control by agents and mechanisms as yet incompletely understood.

Several species exhibit a rise in acetyl CoA carboxylase activity during late pregnancy and early lactation, and immunotitration of the enzyme in rat mammary tissue has shown this to closely match the increase in actual amount of enzyme protein (Mackall and Lane, 1976). Studies with explants of rat, mouse and rabbit mammary tissue *in vitro* have shown that such induction can be mimicked by the addition of prolactin, insulin and (except in rabbit) cortisol. In particular, prolactin not only enhances the synthesis of new enzyme, but also retards its destruction (Mayer, 1978). Such studies on explant culture, together with studies on intact animals, give a picture of a constellation of lipogenic enzymes (pyruvate dehydrogenase, ATP-citrate lyase, acetyl CoA carboxylase, fatty acid synthetase, malic enzyme) being induced at the end of pregnancy by the withdrawal of progesterone and the presence of suitable levels of 'lactogenic' hormones prolactin, cortisol and insulin. However, whereas this appears to be sufficient for the induction of fatty acid synthetase, the induction of ATP-citrate lyase and acetyl CoA carboxylase, and of the lipogenic pathway as a whole, requires that the mother be suckled by the newborn. Therefore, there appears to be some overriding factor still to be identified (Martyn and Hansen, 1980, 1981).

Nutritionally induced changes in fatty acid synthesis

Rates of mammary fatty acid synthesis can be followed in the intact, anaesthetized, laboratory animal by the incorporation of ^3H_2O, which measures the synthesis from acetyl CoA arising from any source, and of [^{14}C]-glucose, which measures synthesis from glucose-derived acetyl CoA. In the fed animal, glucose provides about 60% of the carbon atoms (A.M. Robinson *et al.*, 1978; Williamson *et al.*, 1983; Bussmann *et al.*, 1984; Kuhn and Fisher, 1989). [^{14}C]acetate has been a useful precursor for some studies *in vitro*, but is not suitable for assessing either absolute or physiological rates. The *in vivo* techniques have revealed an extraordinarily close link with the maternal nutritional status. The rates decline within hours of food withdrawal in the rat (within 1 h in the mouse), and cease altogether by about 15 h. On refeeding, lipogenesis restarts within a few minutes, reaches 'fed' values by about 2 h, and overshoots by 5 h. These changes, too, are considerably faster in the mouse. Streptozotocin, an inhibitor of insulin secretion, blocks the return of lipogenic activity. Such large and rapid fluctuations, in a process that probably consumes about half the available body glucose, are clearly essential to ensure the survival of the mother in times of food shortage. In a similar manner, natural diurnal fluctuations in food intake (about a threefold decline between early morning and midday in rats) are accompanied by similar fluctuations in mammary lipogenesis (also about threefold, but with some delay). This must underlie much of the marked diurnal variation in

maternal metabolic rate, judged by heat output, that so closely follows the changing food intake (Brody *et al.*, 1938; Munday and Williamson, 1983). A rise in the fat content of the diet also leads to a decline in mammary lipogenesis without, however, alteration of the chain-length profile of the newly synthesized fatty acids (Grigor and Warren, 1980; Agius and Williamson, 1980).

The very striking nature of these physiological responses raises the question of their mode of control (Williamson, 1990). Plasma insulin is evidently closely involved, and some authors believe that a suitably sensitive response of the mammary gland to the two- or threefold changes in plasma insulin that have been documented might account for the control (A.M. Robinson *et al.*, 1978; Girard *et al.*, 1987). However, the possibility remains that some other hormone plays a role, although this is not glucagon, for which there are no receptors, and probably not adrenaline. The suggestion has been made that a gut hormone may influence mammary sensitivity to insulin (Williamson *et al.*, 1983). Presumably, such a hormone would be released by the presence of digestible carbohydrate in the intestine, since this appears to be the critical nutritional feature (Bussmann *et al.*, 1984; Mercer and Williamson, 1987).

Within the cell, in response to the nutritional signal, short-term control of lipogenesis is probably exerted more or less simultaneously at multiple points, namely glucose transport, phosphofructokinase, pyruvate dehydrogenase and especially acetyl CoA carboxylase. These all represent critical control points on what is really one long pathway for the conversion of glucose into fatty acid. It appears to be a general principle of pathway control that several different points are under coordinate control.

Recently, much attention has been given to the way in which the activity of acetyl CoA carboxylase is regulated. It undergoes activation in the presence of citrate, associated with polymerization into long threads (Ahmad *et al.*, 1978), and inactivation by long-chain acyl CoA, associated with depolymerization. Inasmuch as citrate is a precursor of acetyl CoA in the cytosol, these effects may represent feedforward activation and feedback inhibition. However, caution is due, as it is not yet evident that changes in the concentrations of these allosteric effectors are in fact employed by the cell to bring about alterations in enzyme activity. Therefore, the present focus of attention is upon the inactivation of the enzyme by ATP-dependent phosphorylation, and its reactivation by phosphatase-catalysed dephosphorylation. In rats starved for 24 h, rapidly isolated acetyl CoA carboxylase has been found to carry more phosphate, and to exhibit a lower V_{max} and decreased affinity for citrate. These changes were reversed after 2½ h of refeeding. Both cyclic AMP-dependent and 5′-AMP-dependent protein kinases are known to be able to phosphorylate the acetyl CoA carboxylase of mammary gland with accompanying inactivation. The latter is itself controlled by phosphorylation and dephosphorylation (Munday and Hardie, 1987; Ottey *et al.*, 1989) and is identical with β-hydroxy-β-methylglutaryl CoA reductase kinase (Carling *et al.*, 1989). Yet, striking though these changes are, it is not clear that they account for the reduced rate of lipogenesis over short periods of food withdrawal or in the streptozotocin-treated rat (Munday and Hardie, 1986). It may be that this is achieved rather through rapidly acting allosteric agents (Haystead and Hardie, 1986). There are many puzzles still remaining in the area of regulation of fatty acid synthesis, and the next few years will almost certainly bring forth surprises.

12.6.6 CONTROL OF METABOLIC FLOW

Metabolic balance studies, especially those employing radiochemical tracers, have given a fairly clear picture of how nutrients entering the gland are shared between different

pathways, even if this information as yet pertains to only a few species. About 90% of all long-chain fatty acids become esterified to triacylglycerol and the remainder to phospholipid (Stein and Stein, 1967). Amino acids are used primarily for the synthesis of milk protein, but certain types are converted into others in order to make up for a shortfall. A very interesting case is that of ornithine and arginine, which are used partly to generate extra proline required for synthesis of the caseins. The incidental consequence is that the mammary gland is the only organ other than the liver to produce substantial amounts of urea (Mepham and Linzell, 1967). Arginase appears in the rat mammary gland relatively late in lactation, when we may presume that supplies of proline become limiting (Folley and Greenbaum, 1947; Yip and Knox, 1972).

With glucose, and in ruminants acetate also, multiple fates are most evident and the question of metabolic steering arises. The flux of glucose carbon along the glycolytic pathway provides glycerol phosphate for milk fat formation, and pyruvate for both oxidation to yield ATP and acetyl CoA for lipogenesis. Glucose is also used for the synthesis of lactose, and in the pentose phosphate cycle to generate NADPH for lipogenesis. The other product of this cycle, glyceraldehyde phosphate, re-enters the glycolytic pathway in the case of the rat. In the ruminant, however, in which appreciable levels of the gluconeogenic enzyme fructose bisphosphatase occur, it is probably recycled to hexose phosphate in order to conserve carbohydrate (Crabtree *et al.*, 1981). In those species where sialyllactose is a significant milk sugar, glucose also provides the *N*-acetylglucosamine from which the sialyl moiety is synthesized. The different fates of glucose, together with pertinent original references, have been summarized (Kuhn, 1978). Similar metabolic decisions are taken in the case of acetate, which is also employed both as a lipogenic precursor and as a source of ATP. In ruminants, acetate largely supplants glucose as a source of fatty acids and ATP, leaving it to supply only lactose, glycerol phosphate and the pentose phosphate cycle. Glycolysis is therefore a relatively insignificant pathway. By contrast, in the rat, the importance of glucose as a fatty acid and ATP precursor, together with the lower concentration of lactose in the milk, combine to make glycolysis a highly significant route of metabolism, followed in importance by the pentose phosphate cycle.

Flux control by supply and demand

The proportions of glucose or acetate that are selected for oxidation to give ATP are relatively easily explained. Studies of energy metabolism in tissues generally make it quite clear that ATP is rarely limiting and that energy is supplied according to demand. In the lactating mammary gland, therefore, the major ATP-consuming processes of protein and fat synthesis (lactose synthesis is less important) stimulate the ATP-generating reactions of oxidative phosphorylation through their provision of ADP. The constancy of ATP/ADP ratios in mammary tissue before and after lactogenesis is an indication of the tight coupling and ample capacity of such respiratory control. Elaborate mechanisms exist to ensure adenine nucleotide homeostasis (Atkinson, 1977). A further demand on energy must arise from ion pumping across the plasma membrane, and perhaps also across the apical and secretory vesicle membranes, but these have not yet been assessed. Nevertheless, the pronounced increase in mammary oxygen consumption that is associated with the onset of lactation gives some idea of the extra energy demands made by the synthetic processes of the gland (Folley and French, 1949; A.J. Davis *et al.*, 1979).

A further good example of supply and demand is that of NADPH provision by the pentose phosphate cycle for the purpose of fatty acid synthesis. It has long been accepted

for lipogenic tissues generally that the cycle is essentially driven by the demands of lipogenesis and not, as previously held, the other way round. Glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the cycle, is inhibited by the high prevailing NADPH/NADP ratio of the cytosol. Utilization of NADPH by the reductive steps of fatty acid synthesis acts to relieve this inhibition and facilitate further operation of the cycle. The two processes form a tightly coupled system. The actual mechanism by which this inhibition by NADPH is achieved is complex, however, and has stimulated recent debate.

Internal metabolic homeostasis

At any given level of metabolic activity, a web of cooperative and allosteric interactions operates to coordinate different parts of the metabolic machinery in such a manner as to establish a homeostatic system of great efficacy. Thus, the inhibition of acetyl CoA carboxylase by long-chain acyl CoA ensures that lipogenesis does not exceed the capacity for esterification. The elevated levels of malonyl CoA that characterize active lipogenesis inhibit acyl CoA–carnitine acyltransferase, the rate-limiting enzyme on the pathway of fatty acid oxidation. Conversion of mitochondrial acetyl CoA into citrate relieves an inhibition of pyruvate dehydrogenase, whereas the withdrawal of cytosolic citrate for use as a lipogenic precursor relieves an inhibition of the key glycolytic enzyme phosphofructokinase. The activation of this enzyme, to replenish the precursors of citrate, in turn depletes levels of glucose 6-phosphate and thereby relieves an inhibition of hexokinase. With the exception of pyruvate dehydrogenase control by acetyl CoA, all these feedback inhibitions are mediated by the rapidly reversible binding of a metabolite to an allosteric site on the target enzyme.

It is presumed that excessive accumulation or depletion of metabolic intermediates is avoided by virtue of the binding strength, and the effect of binding upon enzyme activity, that each allosteric site has evolved. Understanding of this sort of homeostatic system, based upon a view of rapid equilibration of free with enzyme-bound metabolites, is fairly well advanced at a qualitative level. The quantitative reconstruction of such systems, however, is still at an early stage (Crabtree and Newsholme, 1987; Kacser and Porteous, 1987; Fell, 1992). It is not clear how far this viewpoint is compatible with the rather different one of metabolites being channelled, in some cases, directly from one enzyme to an adjacent one (Srivastava and Bernhard, 1986). In either case, however, there is second-by-second control.

Long-term inductive control

At the other time extreme, those changes in mammary activity that operate over several hours or even days are probably largely due to changes in amounts of enzymes. Numerous studies have shown that the total assayable amounts of such critical enzymes as lipoprotein lipase, phosphofructokinase, pyruvate dehydrogenase, acetyl CoA carboxylase, glucose-6-phosphate dehydrogenase and lactose synthetase increase during lactation with a pattern closely matching the increase in milk solid production (R.L. Baldwin and Yang, 1974; Kuhn, 1983). In a few cases, changes in enzyme activity have been related to changes in immunologically titratable protein or in actual rates of synthesis of enzyme protein (Mackall and Lane, 1976; Lobato *et al.*, 1986). Some enzymes that are expected to make less contribution to metabolic control, although equal contribution to catalytic activity, also show striking variations. Into this category fall 6-phosphogluconate dehydrogenase, malic enzyme and nucleoside diphosphokinase. Other enzymes change less markedly, for example fatty acid synthetase, phosphoglucomutase or ATP-citrate lyase.

A comparative study of milk composition in different species reveals what must be widely varying rates of biosynthetic pathways. For example, the lactose–fat ratio can vary enormously between species (Jenness, 1974; Bussmann *et al.*, 1984). Almost certainly these gross differences reflect different relative amounts of pathway enzymes, but curiously no one appears to have undertaken the cross-species survey that would be required to establish this.

Reversible short-term control

Profound changes in rates of milk solid synthesis can be observed within a few minutes of administering insulin, or of refeeding starved rats or mice. In well-fed lactating mice the removal of food for as little as 1 h reduces lipogenesis to less than half of that in continuously fed mice. It is not known how the rapid changes mediating this nutritional sensitivity are effected.

For many years it seemed likely that changed amounts of the second messenger cyclic AMP would account for these effects. Several authors were able to report changes in its tissue content with different stages of lactation, or else changes in rates of metabolism following exposure of tissue preparations to cyclic AMP or to its dibutyryl derivative. A role for cyclic AMP seemed all the more likely as the control of acetyl CoA carboxylase through reversible phosphorylation was uncovered (McNeillie *et al.*, 1981). In particular, this enzyme could be phosphorylated by added cyclic AMP-dependent protein kinase, with accompanying loss of activity. The control of lipogenesis by such a mechanism is well established in adipose tissue. However, tissue responses to added cyclic AMP have not been very striking, and much of the cyclic AMP of whole tissue may in fact be extracellular and due to milk. These problems have been reviewed by Clegg (1988). Further, although changes in the phosphorylation state of acetyl CoA carboxylase undoubtedly do occur, and make an important contribution to lipogenic control, other protein kinases can phosphorylate it in the absence of cyclic AMP (Munday and Hardie, 1984, 1986; Ottey *et al.*, 1989; Carling *et al.*, 1989).

The difficulty in accepting cyclic AMP as a second messenger that mediates acute nutritional effects has been pinpointed by recent experiments with acini of rat mammary tissue. Agents known to activate adenylate cyclase produced little elevation of cellular cyclic AMP concentration unless an inhibitor of its breakdown was also added (Clegg and Mullaney, 1985). Further, even when levels of cyclic AMP were experimentally raised, there was no inhibition of lipogenesis and no phosphorylation of acetyl CoA carboxylase (Clegg *et al.*, 1987). Activation of cyclic AMP-dependent kinase was not matched by change in activity of acetyl CoA carboxylase (Clegg, 1988; Clegg and Ottey, 1990).

Insulin is the only hormone that has repeatedly been shown to exert large and rapid effects upon mammary function *in vivo*. Within minutes it enhances glucose uptake and lipogenesis, and activates the mitochondrial enzyme pyruvate dehydrogenase and the cytosolic acetyl CoA carboxylase. The compartmentation of pyruvate dehydrogenase makes it unlikely to be activated directly by cyclic AMP. It is unfortunate that, in attempts to study the action of insulin *in vitro*, it has been found necessary to use concentrations that exceed physiological values by 1000- to 10 000-fold to achieve even quite minor stimulations. It must then be a matter of some doubt whether this is useful. For the most part, tissue preparations of mammary tissue taken out of the body have been remarkably unresponsive to insulin in the short term.

Increasingly, these disappointments in attempts to unravel the nature of the acute control of mammary metabolism suggest one of two possibilities. One is that mammary tissue is an especially delicately poised sys-

tem, the disturbance of which *in vitro* renders it insensitive to insulin or cyclic AMP. In favour of this is the fact that rates of carbohydrate utilization *in vitro* no longer distinguish between the fed or starved status of the donor animal, whereas *in vivo* such rates differ by up to 10-fold. The other possibility is that control of metabolism is really due to a web of different intracellular signals, of which cyclic AMP is only one, and perhaps in the mammary gland is not an important one. Recent research in the area of intracellular signals mediating hormone action increasingly seems to support this idea, and nowhere more so than in the actions of insulin, which may not only utilize novel signal substances but in some actions no simple second messenger at all (Haystead and Hardie, 1986; Saltiel and Cuatrecasas, 1986).

It is notable that the role of cyclic AMP in the hormonal control of carbohydrate and fatty acid metabolism is best established in liver and adipose tissue, where the anabolic action of insulin is balanced by the catabolic actions of other hormones such as glucagon, adrenaline and cortisol. In lactating mammary gland, however, there is no good evidence that any such hormone balance exists, and it may be that the anabolic action of insulin is counterbalanced only by a negative feedback from accumulating milk. It therefore seems important that attention should be given to other ways of mediating hormonal influence. Prolactin has recently been reported to raise concentrations of inositol 1,4,5-trisphosphate in mouse mammary explants (Etindi and Rillema, 1988). This substance is now widely accepted as the major intracellular signal for the release of internal stores of Ca^{2+}, an ion that has in turn long been associated with the activation of secretory tissues. Thus the problem of the acute control of mammary metabolism may become solved as the scope of investigation widens beyond that of cyclic AMP.

12.7 SECRETORY PROCESSES

12.7.1 GENERAL

When, in the nineteenth century, mammary tissue was first ascribed an active role in elaborating milk, microscopists vied with each other in proposing mechanisms for the secretory activity of the epithelial cells. Virchow's notion that milk resulted from complete cellular degeneration (holocrine secretion) was supplanted by Heidenhain's view that only the cell apex was lost (apocrine secretion). The later claim that there is no cytoplasmic loss (merocrine secretion) found firmer support in electron microscopical studies in the 1960s (see Mepham, 1986). Latterly, however, the question has become more complex because no single process appears capable of accounting for secretion of all the different milk constituents.

Major advances in understanding secretion (here defined as the passage of milk constituents from the mammary epithelium to the alveolar lumina) were achieved in the 1960s, a point well made by comparing Linzell's (1959) review and that he wrote with Peaker (Linzell and Peaker, 1971). The progress made is largely attributable to the use of a wide range of investigative techniques, such a autoradiography, histochemistry, isotopic tracers and electrical potential difference (PD) measurements, as well as to refinements in electron microscopy (EM).

It is useful in describing milk secretion to identify five routes by which different milk constituents enter the alveolar lumina, i.e. four transcellular modes, namely the Golgi route, fat secretion, monovalent ion secretion and serum protein secretion; and the paracellular pathway. For several reasons, both biochemical and physicochemical, there are likely to be interactions between the separate modes, but the classification provides a convenient framework for discussion.

12.7.2 THE GOLGI ROUTE

Post-translational modification of mammary-synthesized milk proteins occurs in vesicular elements of the Golgi apparatus. Subsequently, the proteins, frequently in micellar form, are packaged into secretory vesicles, formed at the *trans* face of the Golgi apparatus, which then move towards the apical pole of the cell by a process thought to involve cytoskeletal elements (see Loizzi, 1987). The synthesis of lactose in Golgi vesicles is considered to be responsible for the swelling of secretory vesicles through osmotic entry of water, but formation of larger vesicles seems also to be partly due to the fusion of smaller 'precursor vesicles' (Mather and Keenan, 1983).

Release of the vesicular contents into the alveolar lumina is achieved by exocytosis. The vesicles have a distinctive coat on their cytoplasmic face which, from electron micrography, appears to react with appropriate receptors on the cytoplasmic face of the apical membrane to produce an area of fusion – a 'pre-exocytotic attachment plaque' (Figure 12.9).

Presumably, this area of fusion becomes eliminated in the process of exocytosis but, probably because the process is very rapid, it has not been possible to discern intermediate stages by EM.

Nevertheless, the secretory vesicle coat material does become incorporated, if only transiently, into the apical plasmalemma (Franke *et al.*, 1976). Calcium, phosphorus and citrate are transported into Golgi vesicles from the cytoplasm and become associated with the contained casein micelles. It thus appears that lactose, milk-specific proteins, calcium, phosphorus and citrate are all secreted by a common path – the 'Golgi complex–secretory vesicle' route. This postulate finds support in the observation that in frequently milked goats receiving intravenous infusions of radioactively labelled milk precursors maximum specific radioactivities for all the above milk constituents occurred 2–3 hours later (Linzell *et al.*, 1976). Since lactose is the major osmole in milk, the route is also likely to be an important avenue for water transfer, but there is uncertainty as to whether osmotic equilibrium is complete prior to exocytosis. Peaker's earlier calculations (1977, 1978) suggested that lactose concentrations in Golgi vesicles were 3–4 times those in milk; later (1983), taking account of possible inaccuracies due to disruption of the mammary epithelium, he considered it safer to conclude that 'the contents of the Golgi vesicles could be at somewhat higher concentration than milk'; in either case, osmotic equilibrium would be completed by water diffusion across the apical membrane.

12.7.3 MONOVALENT ION SECRETION

In experiments in which isotopically labelled sodium, potassium and chlorine were infused intravascularly in frequently milked goats (Linzell *et al.*, 1976), maximum specific radioactivities in milk were achieved within 1 h, suggesting that the transfer process involved differs from the Golgi route described above.

The most widely accepted theory for monovalent ion secretion is that proposed by Linzell and Peaker (1971). It is based largely on PD determinations, tracer studies and measurements of concentrations of ions in ECF, intracellular fluid (ICF) and milk. In the transcellular process (i.e. involving passage across both basolateral and apical membranes of the cell), K^+ and Na^+ are distributed between ICF and milk in accordance with the PD across the apical membrane. The concentrations of Na^+ and K^+ are lower in milk than in the ICF but the ratio between them, 1:3 for most species, is similar in both fluids. Since Golgi-derived secretory vesicles appear to become incorporated into the apical membrane, it is postulated that K^+ and Na^+ are transferred into their lumina by the same process.

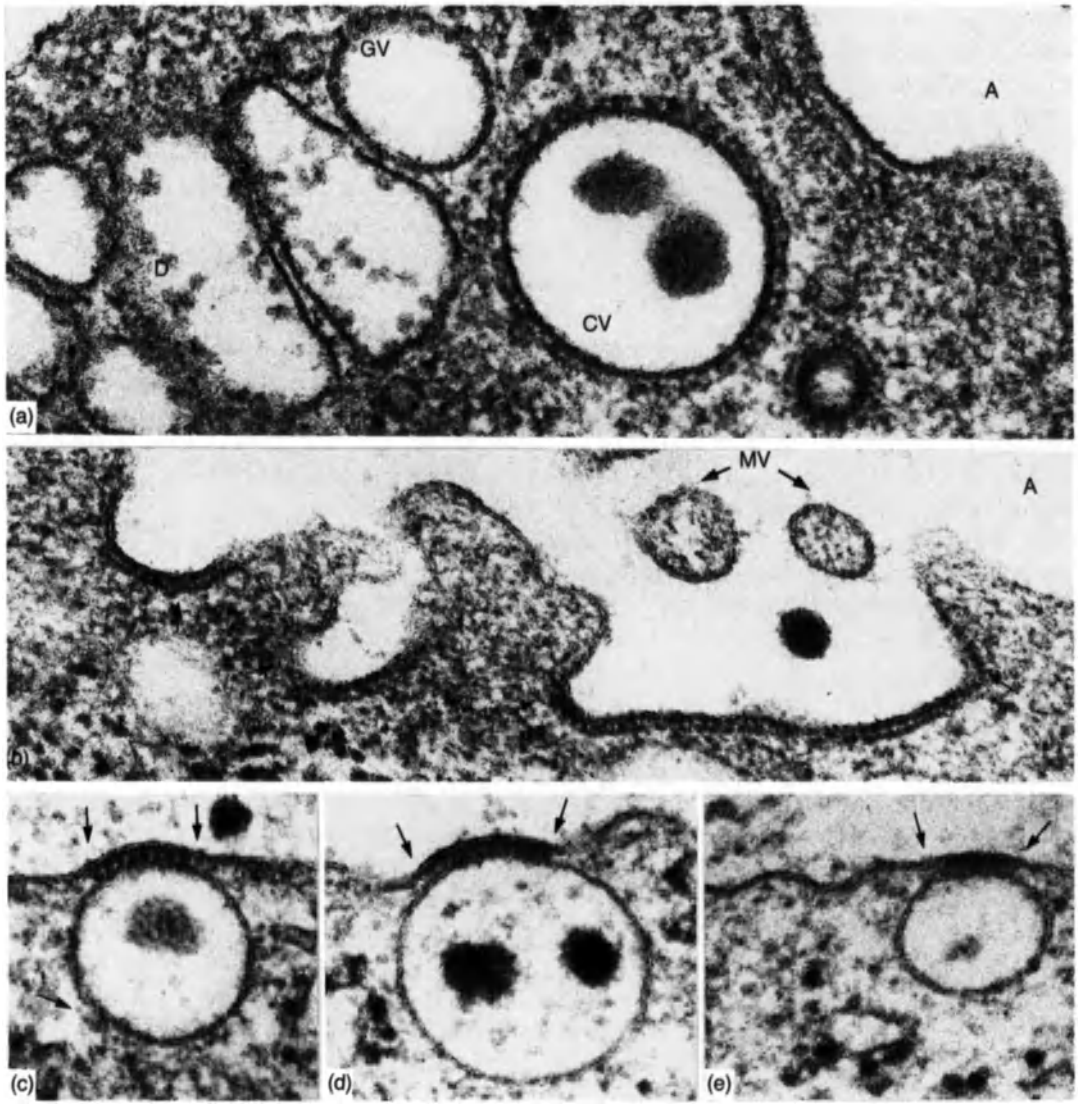


Figure 12.9 Electron micrographs of the apical region of secretory cells in lactating mammary tissue of a rat showing processes involved in the secretion of casein micelles. (a) The major types of vesicle are identified; GV, Golgi apparatus-associated vesicles with a smooth surface; CV, coated vesicles containing casein; arrow, smaller vesicles also coated on the cytoplasmic face. A = alveolar lumen (magnification ×97 750). (b–d) Pre-exocytotic attachment plaques forming between secretory vesicles and apical plasma membrane (short arrows): coated material is still present on some vesicles (long arrow in b) (magnifications: b ×85 000; c ×89 250; d ×93 500). (e) Integration of coat material of secretory vesicle membrane into apical plasma membrane (arrows); microvilli (MV) appear in cross-section (magnification ×95 000). (Reproduced from Franke *et al.*, 1976, with permission.)

The ICF concentrations of these ions are determined by a Na^+-K^+-activated ATPase, which histochemical studies indicate is confined to the basolateral surfaces of the cell (see Peaker, 1978).

The secretion of Cl^- does not, however, fit so readily into this scheme. Since both the electrical and chemical gradients would drive Cl^- from the cell into milk, it has been proposed that its low concentration in milk may be explained by reabsorption across the apical membrane, although other explanations might apply (Peaker, 1983). Thus, according to this hypothesis, milk concentrations of K^+ and Na^+ are maintained at low levels by the apical PD. Peaker's (1983) studies suggest that the PD is water-flow induced, in consequence of osmotic equilibration following lactose synthesis within the Golgi vesicles. Confirmatory evidence is provided by the decreased apical PD, with associated increases in milk K^+ and Na^+ concentrations, which resulted when isolated perfused goat mammary glands were deprived of a glucose supply (Peaker, 1977).

However, not all results obtained are consistent with the above scheme. For example, the studies of Berga and Neville (1985) on mouse mammary tissue suggest that the K^+/Na^+ ratios in intracellular fluid and milk are not the same. These authors postulated the existence of an active transfer system for Na^+ and/or K^+ at the apical membrane.

12.7.4 PARACELLULAR ROUTE

Whatever the precise nature of the transcellular route, a complicating factor in any definition of monovalent ion transfer to milk is the extent to which passage may occur **between** cells (the paracellular route). Most commonly, paracellular transfer is associated with the prepartum and involuting gland, but it may also occur in conditions in which the mammary epithelium is stretched (e.g. when the alveoli are distended with milk) or when the effectiveness of tight junctions is otherwise impaired (e.g. in mastitis). In such circumstances milk concentrations of Na^+ and Cl^- rise and those of K^+ and lactose fall, i.e. the gradients established by transcellular processes are partially offset by diffusion between the ECF and milk via leaky tight junctions (section 12.8.4). Depending on the degree of leakage, large molecules may also be involved, e.g. in late pregnancy α-lactalbumin may be detected in blood.

12.7.5 SECRETION OF MILK FAT

Autoradiographic studies showed that, within minutes of their intravenous injection, radioactively labelled fatty acids became associated with fat droplets in the mammary secretory cells (Stein and Stein, 1967). EM evidence suggests that TG molecules can be rapidly transferred, in the form of 'lipovesicles', to existing globules as they migrate from their sites of synthesis in endoplasmic reticulum towards the apical membrane (see Mather, 1987). In contrast, the appearance of isotopically labelled fat in secreted milk was only reached a maximum 5–7 h after the intravenous injection of fat precursors into goats (Linzell *et al.*, 1976). This is a much longer delay than for monovalent ions, lactose and proteins, and is indicative of a distinct secretory process.

It is presumed that, as for Golgi-derived secretory vesicles, cytoskeletal elements are important in intracellular migration of fat droplets, but it is transfer across the apical membrane which has attracted most attention, and indeed debate. According to the theory first proposed by Bargmann and Knoop (1959), as fat droplets reach the cytosolic face of the apical membrane they become enveloped by the latter, which eventually completely surrounds the 'pinched-off' fat globules present in the alveolar lumina (Figure 12.10).

Wooding (1977), on the other hand, claimed that fat droplet secretion depends on secretory vesicles, which by encircling the fat

droplets prior to their own fusion with the apical membrane effect fat secretion by a process which is, at least partly, exocytotic. Wooding denied the efficacy of 'pinching off' as an active secretory process, although admitting that milk fat globule membrane (MFGM) may acquire contributions from both secretory vesicles and apical sources.

Whether or not both apical and secretory vesicle membranes are involved, the nature of the attractive forces between them and the lipid droplets, which accomplish secretion of the latter from the cell, is a matter of considerable interest. As the cytosolic droplets come into contact with these membranes, a uniform dense layer of proteinaceous material (10–50 nm thick) is formed between the two surfaces so that in the MFGM of the secreted globule it is possible to identify four layers, namely, from the core outwards: (i) an

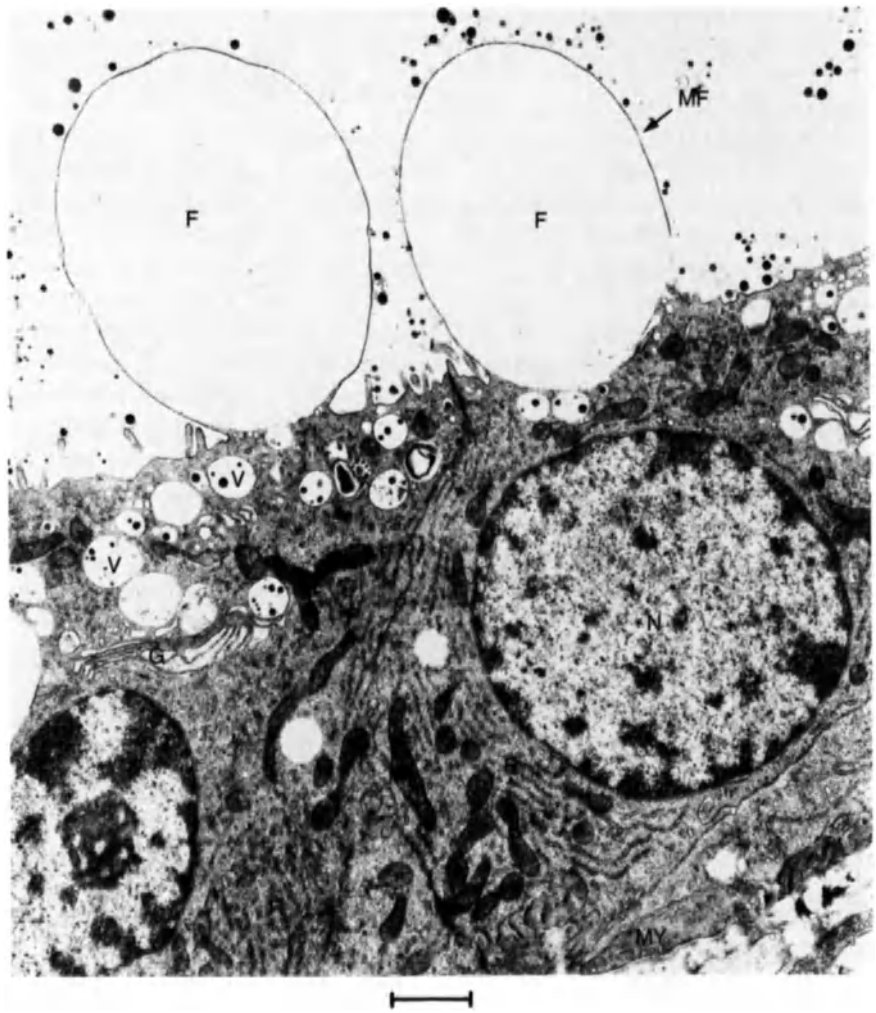


Figure 12.10 Electron micrograph showing secretion of fat droplets by two secretory cells in ovine mammary tissue (scale bar 1.0 μm). F, fat droplets; MF, milk fat globule membrane; V, secretory vesicle containing protein granules; N, nucleus; G, Golgi apparatus; R, rough endoplasmic reticulum, M, mitochondria; MY, myoepithelial cells. (Courtesy of Dr F.B.P. Wooding.)

amorphous layer of material derived from lipovesicles (<1 nm); (ii) the protein coat (10–50 nm); (iii) the lipid bilayer (10 nm); and (iv) the glycocalyx (<1 nm), a complex of glycosaminoglycans and oligosaccharide chains (Mather, 1987). The proteins of the second layer include butyrophilin and xanthine oxidase. The former is a mammary cell-specific glycoprotein which appears specifically located in the apical plasmalemma, the latter an enzyme distributed widely throughout the cell. The origin of elements of MFGM is thus diverse, and attempts at closer definition are complicated still further by EM evidence that intramembranous particles are cleared from the lipid bilayer during the process of secretion (Mather and Keenan, 1983).

Usually, globules of fat in alveolar lumina are simply surrounded by MFGM, although membrane material may subsequently be shed by a process of vesiculation (Wooding, 1977). In some cases, however (e.g. about 5% of fat globules in goat's milk), the globules have attached crescents of cytoplasm, often containing secretory vesicles and endoplasmic reticulum. It is surmised that these so-called 'signets' result from premature secretion caused by the mechanical stresses to which some cells may be exposed during milk ejection (Linzell and Peaker, 1971).

12.7.6 SECRETION OF IMMUNOGLOBINS

Ig molecules may either be derived from maternal plasma (e.g. IgG) or synthesized locally in the mammary gland by plasma cells in the lamina propria. In both cases secretion appears largely to involve transcellular passage following endocytosis. IgG1, though present in bovine plasma at roughly equivalent concentrations to IgG2, is highly concentrated in colostrum and milk. It is believed that IgG1 molecules bind, via their Fc domains, to specific receptors on the basolateral membranes of secretory cells and, following endocytosis, traverse the cytoplasm within vesicles (Lascelles, 1977).

The membrane receptors for polymeric Ig (e.g. IgA and IgM) transfer have been studied quite extensively. Following synthesis in the secretory cell, the receptor (a glycoprotein) is sorted to the basolateral membrane, where it binds to its polymeric Ig ligand. The receptor–ligand complex is then endocytosed and the endoplasmic vesicle shuttled to the apical membrane. Secretion of the complex, e.g. sIgA, involves exocytosis, but also cleavage of the receptor 'membrane anchoring domain' (Solari and Kraehenbuhl, 1987).

12.7.7 MEMBRANE FLOW

It is apparent that several of the secretory processes discussed involve either loss of membrane material from the cell (as in fat secretion) or transfer of membrane material from one cell organelle to another. Such observations imply that there is a continuous flow of membrane through the cells with selective additions and deletions of specific molecules occurring as membrane traverses the route: endoplasmic reticulum → *cis* Golgi vesicles → *trans* Golgi vesicles → secretory vesicles → apical membrane → MFGM. Moreover, it is likely that not only does membrane material flow, but that it is also recycled, since calculations for bovine mammary tissue indicate that the **net** gain of apical membrane from secretory vesicle fusion greatly exceeds the loss in MFGM. Reviews of research in this field are provided by Mather and Keenan (1983) and Mather (1987).

12.8 CONTROL OF MILK SECRETION

12.8.1 GENERAL

The factors modulating the rates of secretion of the various components of milk are clearly of paramount importance in terms of its adequacy in meeting neonatal nutritional requirements. For most, if not all, biological activity, two types of factor may be ident-

ified, often euphoniously categorized as 'nature' and 'nurture'. In the present context these might be expressed as 'genetic potential' and 'maternal nutrition', or more generally as 'endogenous' and 'exogenous' factors. Milk secretion is governed by the interaction of factors of these two types.

Earlier sections of this chapter have discussed numerous control points in the causal chain from food intake to milk output, including the metabolic priorities of the whole body, the substrate supply to the glands, carrier-mediated substrate access to the cell interiors and the subsequent synthetic processes involved in elaborating milk constituents. Chapter 11 deals with the predominant role of the endocrine system in controlling these processes. The object of this section is to summarize non-endocrine control factors operating at the level of the mammary epithelium which have not so far received attention.

One or two points are of wide applicability. Within a given species, genetic and environmental factors, together with limitations imposed by physicochemical determinants, act as significant constraints to fluctuations in milk composition. By and large, if milk secretion is proceeding, its composition will be fairly characteristic of the species concerned, at that particular lactational stage. This implies that the ultimate control of milk secretion may be subject to a single, or few, pivotal factor/s.

A second, rather self-evident, point is that secretory rate depends on the product of an intensity factor (the activity of the cells) and an extensity factor (their total number). The former is often gauged by the parenchymal RNA/DNA ratio, the latter by the total parenchymal DNA (i.e. DNA_t) (see Knight, 1984). Since secretory activity is reflected in the degree of differentiation of the cells, increases in the intensity and extensity factors correlate with cellular hypertrophy and hyperplasia respectively. Thus, increased milk output might be attributable to hypertrophy, hyperplasia or both processes together; decreased milk output to reduced cellular activity and/or reduced cell numbers, with necrotic cells not being replaced.

12.8.2 AUTOCRINE REGULATION

A unifying theme in these considerations is the concept of autocrine regulation. It has long been known that frequent milking of dairy animals leads to increased yields. For example, thrice-daily milking of cows (Archer, 1983) and goats (Henderson *et al.*, 1985) increased yields about 20% and 30%, respectively, above those obtained on the normal twice-daily routine. That this is not due to enhanced secretion of galactopoietic hormones is indicated by the fact that frequent unilateral milking (one gland only) of goats (Henderson *et al.*, 1983) and cows (Hillerton *et al.*, 1990) produces an increased yield only in that gland. While excessive physical distension of alveoli can reduce milk secretion, relief of that condition in frequently milked glands was not the explanation of the increased yields because it was achieved even when the milk removed was immediately replaced by an equal volume of isosmotic sucrose solution (Henderson and Peaker, 1984). Such observations led to the formulation of the hypothesis that enhanced yields in these experiments were due to more frequent removal of a locally active inhibitor present in the milk (Wilde and Peaker, 1990). Its inhibitory action has been demonstrated with mammary explants from mid-pregnant rabbits (Wilde *et al.*, 1987). In this bioassay system, progressive purification of the active agent was signalled by increasingly sensitive inhibition of lactose and casein synthesis. The inhibitor has been characterized as a heat-labile constituent of the serum protein fraction of milk, with a molecular weight of 10 000–30 000 daltons. It appears to act by negative feedback across the apical membranes of the mammary epithelium, and the dose dependence of the response indicates

that inhibition is determined by the concentration of the substance in the alveolar lumina (Knight *et al.*, 1988).

Whilst definitive identification of the putative autocrine regulator is lacking, there seems little doubt as to its importance in exercising fine control over the energetically expensive demands of milk secretion, such that supply is closely tailored to 'need'. This is perhaps best illustrated in species where a fixed 'teat order' prevails, e.g. pigs. A consideration which merits more attention than it has so far received is the necessity for an **increase** in inhibitory action with time if the galactopoietic effect of frequent milking is to be explained in terms of this autocrine regulator: the mere presence of the inhibitor in milk at constant concentration would not produce the observed results. Two types of factor can be identified (not mutually exclusive) which might transform a constant into an incremental inhibition: (i) a progressive increase in the intraluminal activity of the agent and (ii) a progressive increase in the sensitivity of the mammary epithelium to the agent. Conditions leading to (i) might include progressive increases in the rate of inhibitor secretion or activation following secretion (e.g. by proteolytic processing) and decreases in the extent of deactivation; conditions promoting (ii) might depend on changes in surface receptors or on conformational changes as cells are stretched due to alveolar filling.

12.8.3 MAMMARY CELL PROLIFERATION

A feature of the autocrine mechanism described is its acute nature. Frequent milking leads to a galactopoietic response within hours and on its discontinuation control yields are rapidly restored. When, however, frequent milking is continued for longer periods several response phases are identifiable (Wilde *et al.*, 1987). Within 10 days of the onset of frequent milking in goats the activity of two key lipogenic enzymes, acetyl CoA carboxylase and fatty acid synthetase, and of galactosyltransferase had significantly increased. But after 13 weeks of unilateral frequent milking, the activities of these enzymes did not differ between the thrice- and twice-milked glands despite the maintained elevation of milk yields in the former. Remarkably, RNA and DNA contents of the glands did not differ, implying that increased yields in the thrice-milked glands were achieved by greater efficiency of secretion from a stable cellular capacity.

In other experiments, 37 weeks after the onset of unilateral frequent milking, when yields in thrice-milked glands were 47% higher than controls, both parenchymal DNA_t and RNA/DNA ratios were significantly increased by frequent milking, whereas the activities of several enzymes were unchanged (Wilde *et al.*, 1987). These results suggest that frequent milking induces a series of distinct response phases: initially, acute autocrine mechanisms predominate, being succeeded by a hypertrophic phase and finally by a hyperplasic phase. Studies with [^{3}H]thymidine indicated that the latter was associated with increased cellular proliferation, but decreased cellular involution may also have been involved.

12.8.4 LOCAL FACTORS AT LACTOGENESIS

Evidence for a relationship between milk removal, cellular differentiation and secretory activity has also been reported for dairy ruminants during the periparturient period. For example, ultrastructural studies of mammary tissue from cows unilaterally milked prepartum showed markedly greater secretory cell Golgi development in milked than in non-milked glands (Akers and Heald, 1978), suggesting that the prepartum secretion is inhibitory to cellular differentiation. On the other hand, Linzell and Peaker's (1974) study of compositional changes in secreted ions of prepartum milked goats led them to propose that the secretions contained a factor which delayed the onset of lacto-

genesis by maintaining a paracellular leakage through the mammary epithelium. Removal of this factor thus led to 'tightening' of cell junctions and establishment of normal milk secretion. The studies on goats of Maule Walker and Peaker (1980) suggested that this local lactogenic inhibitor might be prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is synthesized by the secretory cells. Just before parturition, the normal output of $PGF_{2\alpha}$ into mammary venous blood declines rapidly, whereas its concentration in mammary secretions increases. This implies a redirection of the excretory route for $PGF_{2\alpha}$ from one involving the basal membrane of the cell to that involving the apical membrane. Moreover, at this time the cells develop the capacity to metabolize $PGF_{2\alpha}$ to 13,14 dihydro-15, keto-$PGF_{2\alpha}$ ($DHKPGF_{2\alpha}$), releasing them from the inhibitory action of $PGF_{2\alpha}$ itself. Removal of excess $PGF_{2\alpha}$ in the milk by the neonate sustains this disinhibitory process (Maule Walker, 1984).

There thus appear to be at least two distinct autocrine factors which affect milk secretion at different lactational stages. Others have been proposed; for example, Gonzalez and Verly (1976) isolated a 2000 to 3000-Da compound from mammary tissue, which specifically and reversibly inhibited mammary DNA synthesis. Latterly there has been growing evidence for the involvement of peptide growth factors in control of mammary function, and it has been speculated that some may operate by an autocrine and/or paracrine process (see Dembinski and Shiu, 1987). There seems little doubt that unravelling the interaction between these putative local regulators and the wide array of systemic hormones which modulate mammary function will present formidable problems.

12.8.5 CELL NUMBERS

An important element of the gland's secretory activity may thus depend on its ability to increase or maintain secretory cell numbers during lactation. It has long been recognized that in rats and mice mammary cell proliferation continues post partum, contributing to the increasing milk yield in early lactation. But the realization that mammogenesis and lactation are not mutually exclusive processes in ruminants is relatively recent (Knight, 1984): in at least one dairy species, the goat, a significant increase in mammary DNA_t occurs in the first 3 weeks post partum (Anderson *et al.*, 1981). The natural changes in mammary development during lactation can be exaggerated by frequent milking (as described above) or by hemimastectomy, when compensatory hypertrophy of the remaining gland occurs (e.g. in goats; Knight, 1987). Few studies have been performed to evaluate the relative importance for secretion rate of changes in RNA/DNA ratio and DNA_t in the declining phase of lactation, but in rats reduction in RNA/DNA ratio seems more important (Knight *et al.*, 1984), whereas in goats falling yield is attributable to reduced DNA_t (Knight *et al.*, 1988).

12.8.6 INTERCELLULAR COORDINATION OF SECRETION

Since secretory rate is the resultant of the combined activities of individual cells, it is important to take account of controls which might operate at the supracellular level. The best-established factors are, of course, oxytocin and other humoral agents which stimulate myoepithelial cell contraction (Chapter 11), but the possibility of other mechanisms merits consideration.

Leaving aside pathological states, it is apparent that there is considerable cytological heterogeneity within even fully lactating glands. Thus, highly differentiated and largely undifferentiated cells can coexist in the same gland (Saake and Heald, 1974). On the other hand, within individual alveoli a high degree of secretory synchrony is usually apparent, both using histological techniques and in autoradiographic studies of the transit times of labelled milk precursors through

cells (Heald and Saake, 1972). There are, however, differences in transit times between equally differentiated alveoli both *in vivo* and *in vitro*. It is possible that local differences in intraalveolar pressure are responsible, via the postulated autocrine factor, but it is also conceivable that small molecules or ions (e.g. Ca^{2+}, Baumrucker, 1978) elicit pulsatile discharge of milk components (i.e. 'stimulus–secretion coupling'). If such secretory cycles were sufficiently rapid and temporally overlapping in different regions of the gland the outcome would produce the observed, fairly stable, secretory rate. The coordination evident between cells in a single alveolus is probably effected by agents permeating gap junctions (section 12.3.2).

12.9 TRENDS AND SIGNIFICANCE OF RESEARCH ON LACTATION

We conclude this chapter with a short survey of current trends in research on lactation, together with an assessment of the importance of present and future developments.

Lactation, directly or indirectly, plays a fundamental but largely unrecognized role in human welfare. In nutritional terms, breast milk provides totally for the requirements of younger infants, while animal milk does so significantly for many millions of adults. Lactation in non-dairy animals may also be vital in the rearing of livestock used for meat, and for countless other purposes, such as transport and draught power. In economic terms, milk is a major commodity, a fact which stems from the highly diverse nature of dairy products, e.g. butter, cheese, yoghurt, kumiss. For the vast majority of women, worldwide, lactation is the principal regulator of fertility, whereas breast cancer is one of the commonest causes of morbidity and mortality in the West. It is thus not surprising that most research on lactation is perceived as having foreseeable practical applications. But in addition, mammary tissue has proved to be very suitable material for those pursuing questions of much wider applicability; for example, fundamental studies on biochemical control processes are facilitated by the high metabolic activity of lactating tissue and its exquisite sensitivity to many hormones.

Historically, the medical context took precedence over that of dairying. If we regard von Haller's *Elementa Physiologiae Corporis Humani* (1778) as marking the beginning of modern physiology, for almost a hundred years virtually all scientific treatises which dealt with lactation were concerned primarily with its manifestation in women, e.g. the works of Young (1761) and A.P. Cooper (1840). Scientific interest in milk production in animals only developed with the industrialization of dairying in the latter half of the nineteenth century, leading to the publication of such important books as those by Furstenburg (1868) and Fleischmann (1896), and from then until only very recently the dairying context was dominant in lactational physiology. Recently, in the West, this situation has changed: as a result of milk production outstripping demand there has been a marked, if short-sighted, reduction in investment in dairy research.

Research of significance to human lactation has three principal goals: (i) definition of the physiological roles and control of breastfeeding to allow for prevention and/or correction of dysfunctions; (ii) definition of the composition of breast milk to permit formulation of acceptable substitutes; (iii) elucidation of factors instrumental in the diagnosis, causation, amelioration and cure of breast cancer. There is a sense in which the first two objectives are in conflict; and it is somewhat paradoxical that the commercial motivation to manufacture artificial milk (based on cow's milk) has only served to establish how different the compositions of human and cow's milk are, and how superior breast milk is to any contrived substitute. At a trivial level, attempts to mimic breast milk are reminiscent of Aesop's fable of the tortoise and the hare:

every 'improved' formula is rendered obsolete by deeper understanding of the nature of milk. The more serious aspect is the grave consequences of misapplication of this Western technology in developing countries, and, indeed, in the poorer sections of developed countries (e.g. Mepham, 1989a). Human lactation is discussed in recent books by Jelliffe and Jelliffe (1978), Neville and Neifert (1983), Jensen and Neville (1985), Schaub (1985), Hamosh and Goldman (1986) and Goldman *et al.*, (1987) and in two WHO reports (1981,1985).

Much research on mammary tissue is focused on the third objective above, i.e. tackling the problem of breast cancer, which in Britain and the USA is a common cause of morbidity and mortality in women. Most approaches build on the apparent hormone dependence of the disease (Angeli *et al.*, 1986), although fundamental cytological studies are also of undoubted importance. A link with breastfeeding is indicated by the claim that the incidence of premenopausal breast cancer is inversely related to earlier breastfeeding duration (McTiernan and Thomas, 1986).

Studies on the physiology and biochemistry of lactation form but a small segment of the field of dairy research, which encompasses chemical, microbial and engineering technology as well as agronomic, management and economic aspects of dairy husbandry. In the past, the benefits of physiological research seem to have related most directly to feeding systems for dairy animals and indirectly to procedures adopted in breeding, housing, milking and veterinary treatment. Attempts to manipulate milk yields by endocrine intervention, through mammogenic and galactopoietic responses, have been made for at least 50 years, but it is only recently that the prospect has appeared commercially viable. Large-scale production of bovine growth hormone (or somatotrophin, bST) by genetic engineering techniques, which it is proposed to administer regularly to cows by subcutaneous injection, has been claimed as 'the herald of a new technological era' (D.R. Baldwin, 1987). Critics focus on the undesirable social consequences of introducing a treatment which markedly increases milk yield in countries already producing a surplus (Mix, 1987) and on potential problems for animal welfare (Kronfeld, 1987). Whatever the merit of milk yield-enhancing technology in developed countries, there can be little doubt that appropriate research directed to increasing milk yields in poorer countries holds the promise of substantial development in both nutritional and economic terms (Mepham, 1987b). The problems encountered and strategies for their solution are discussed in the conference proceedings edited by A.J. Smith (1985). For other reviews on the physiology of lactation in dairy animals see the books edited by Larson (1985), Gravert (1987) and Garnsworthy (1988).

Genetic engineering also presents the prospect of exerting control over milk yield and composition in ways not even imagined only a few years ago. For example, growth hormone genes have been injected in porcine ova just following fertilization to produce transgenic animals. Were this technique to be applied successfully to dairy animals, it is possible to envisage a rapid and permanent improvement in productivity in developing countries (Hodges, 1986). However, such optimism could prove unfounded if improvements are not also made in animal nutrition, which is usually the major constraint to increasing milk yields in developing countries (Mepham, 1991).

Appropriate manipulation of other genes could, in theory, be used to effect compositional modification. For example, production of a low-fat milk would be found more acceptable by many in Western countries in which there is concern over the alleged association between ischaemic heart disease and saturated fat intake (Committee on Medical Aspects of Food Policy, 1984), and a

lactose-free milk would be a boon to many millions for whom consumption of raw milk is limited by the condition of lactose malabsorption (Paige and Davis, 1985). A striking demonstration of the capabilities of genetic engineering is the production by lactating transgenic sheep of pharmaceuticals for use in human medicine (Clark *et al.*, 1989). The potential for control by immunological means is suggested by results indicating the importance of autocrine control of milk secretion: isolation of the factor involved, and the raising of an antibody to it, might enable the suppression or stimulation of yields, respectively, according to requirements. Some of the wider issues presented by biotechnological advances in dairying are discussed by Mepham (1989b,c, 1991, 1992).

Another important development of recent years is the study of the energetics of lactation in the context of parental investment. Until they are weaned, young mammals receive all, or almost all, their energy requirements for maintenance and growth from maternal milk. Consequently, litter growth represents a meaningful index of maternal investment. The significance of the post-natal growth phase in mammals lies in the fact that a large proportion of the energy requirements for reproduction in females is associated with lactation (e.g. 75–80% in ungulates). Species survival is thus ultimately dependent on effective partitioning of energy between mother and infants, and elucidation of the means by which this is achieved in different species promises to provide valuable new insights into the evolutionary adaptation of reproductive processes to environmental conditions. The reader is referred to the volume edited by Loudon and Racey (1987) for discussion of these important questions.

REFERENCES

Abraham, S., Hirsch, P.F. and Chaikoff, I.L. (1954) The quantitative significance of glycolysis and non-glycolysis in glucose utilization by rat mammary gland. *Journal of Biological Chemistry*, **211**, 31–38.

Aggeler, J., Chung, C.S. and Bissell, M.J. (1988) Regulation of milk protein and basement gene expression: The influence of the extracellular matrix. *Journal of Dairy Science*, **77**, 2830–2842.

Agius, L. and Williamson, D.H. (1980) Rapid inhibition of lipogenesis *in vivo* in lactating rat mammary gland by medium- or long-chain triacylglycerols and partial reversal by insulin. *Biochemical Journal*, **192**, 361–364.

Ahe, D., Janich, S., Scheidereit, C., Renkawvitz, R. Schütz, G. and Beato, M. (1985) Glucocorticoid and progesterone receptors bind to the same sites in two hormonally regulated promoters. *Nature* (*London*), **313**, 706–709.

Ahmad, F., Ahmad, P.M., Pieretti, L. and Watters, G.T. (1978) Purification and subunit structure of rat mammary gland acetyl coenzyme A carboxylase. *Journal of Biological Chemistry*, **253**, 1733–1737.

Akers, R.M. and Heald, C.W. (1978) Stimulatory effects of pre-partum milk removal on secretory cell differentiation in the bovine mammary gland. *Journal of Ultrastructural Research*, **63**, 316–322.

Alberts, B., Bray, D., Lewis, J., Ruff, M., Roberts, K. and Watson, J.D. (1983) *Molecular Biology of the Cell*, Chapter 7, Garland Publishing, New York.

Amano, J., Messer, M., and Kobata, A. (1985) Structures of the oligosaccharides isolated from milk of the platypus. *Glycoconjugate Journal*, **2**, 121–135.

Anderson, R.R. and Sinha, K.W. (1972) Number of mammary glands and litter size in the golden hamster. *Journal of Mammalogy*, **53**, 382–384.

Anderson, R.R., Harness, J.R., Snead, A.F. and Salah, M.S. (1981) Mammary growth pattern in goats during pregnancy and lactation. *Journal of Dairy Science*, **64**, 427–432.

Angeli, A., Bradlow, H.L. and Dogliotti, L. (eds) (1986) *Endocrinology of the Breast: Basic and Clinical Aspects*, Annals of the New York Academy of Sciences, Vol. 464, New York Academy of Sciences, New York.

Annison, E.F. (1983) Metabolite utilization by the ruminant mammary gland, in *Biochemistry of Lactation*, (ed. T. B. Mepham), Elsevier, Amsterdam, pp. 399–436.

Annison, E.F. and Linzell, J.L. (1964) The oxidation and utilization of glucose and actate by the mammary gland of the goat in relation to

their overall metabolism and to milk formation. *Journal of Physiology (London)*, **175**, 372–375.

Archer, P. (1983) Milking three times a day. *Milk Marketing Board Report 34*.

Atkinson, D.E. (1977) *Cellular Energy Metabolism and its Regulation*. Academic Press, New York.

Babad, H. and Hasid, W.Z. (1966) Soluble uridine diphosphate D-galactose: D-glucose β-4-D-galactosyltransferase from bovine milk. *Journal of Biological Chemistry*, **241**, 2672–2678.

Baile, C.A. and Della-Fera, M.A. (1988) Physiology of control of food intake and regulation of energy balance in dairy cows, in *Nutrition and Lactation in the Dairy Cow*, (ed. P.C. Garnsworthy), Butterworths, London, pp. 251–261.

Baile, C.A. and Forbes, J.M. (1974) Control of feed intake and regulation of energy balance in ruminants. *Physiological Reviews*, **54**, 160–214.

Baker, E.N., Rumball, S.V. and Anderson, B.F. (1987) Transferrins: insights into structure and function from studies on lactoferrin. *Trends in Biochemical Sciences*, **12**, 350–353.

Baldwin, D.R. (1987) Bovine somatotrophin. *Veterinary Record 31.1.87.*, p. 120.

Baldwin, R.L. and Yang, Y.T. (1974) Enzymatic and metabolic changes in the development of lactation, in *Lactation: a Comprehensive Treatise*, Vol. 1, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 349–411.

Bargmann, W. and Knoop, A. (1959) Über die Morphologie der Milchsekretion: Licht und elektronen-mikroskopische Studien an der Milchdrüse der Ratte. *Zeitschrift für Zellforschung*, **49**, 344–388.

Barry, J.M., Bartley, W., Linzell, J.L. and Robinson, D.S. (1963) The uptake from the blood of triglyceride fatty acids of chylomicra and low-density lipoproteins by the mammary gland of the goat. *Biochemical Journal*, **89**, 7–11.

Barthelson, R. and Roth, S. (1985) Topology of UDP-galactose cleavage in relation to N-acetyllactosamine formation in Golgi vesicles. Translocation of activated galactose. *Biochemical Journal*, **225**, 67–75.

Bauman, D.E. and Elliott, J.M. (1983) Control of nutrient partitioning in lactating ruminants, in *Biochemistry of Lactation*, (ed T.B. Mepham), Elsevier, Amsterdam, pp. 437–468.

Baumrucker, C.R. (1978) Calcium transport in lactation, in *Lactation: a Comprehensive Treatise*, Vol. 4, (ed. B.L. Larson), Academic Press, New York, pp. 463–474.

Baumrucker, C.R. (1985) Amino acid transport systems in bovine mammary tissue. *Journal of Dairy Science*, **68**, 2436–2451.

Baumrucker, C.R. and Pocius, P.A. (1978) Gamma glutamyl transpeptidase in lactating mammary secretory tissue of cow and rat. *Journal of Dairy Science*, **61**, 309–314.

Berga, S.E. and Neville, M.C. (1985) Sodium and potassium distribution in the lactating mouse mammary gland *in vitro*. *Journal of Physiology*, **361**, 219–230.

Berliner, L.J. and Robinson, R.D. (1982) Structure–function relationships in lactose synthase. Structural requirements of the uridine 5′-diphosphate galactose binding site. *Biochemistry*, **24**, 6340–6343.

Bhattacharjee, M. and Vonderhaar, B.K. (1984) Thyroid hormones enhance the synthesis and secretion of α-lactalbumin by mouse mammary tissue *in vitro*. *Endocrinology*, **115**, 1070–1077.

Bickerstaffe, R. and Annison, E.F. (1971a) Triglyceride synthesis in goat and sow mammary tissue. *International Journal of Biochemistry*, **2**, 153–162.

Bickerstaffe, R. and Annison, E.F. (1971b) The desaturase activity of goat and sow mammary gland. *Comparative Biochemistry and Physiology*, **35**, 653–665.

Bienkowski, R.S. (1983) Intracellular degradation of newly synthesized secretory proteins. *Biochemical Journal*, **214**, 1–10.

Bingham, E.W. and Farrell, H.M. (1974) Casein kinase from the Golgi apparatus of lactating mammary gland. *Journal of Biological Chemistry*, **249**, 3647–3651.

Bishop, C., Davies, T., Glascock, R.F. and Welch, V.A. (1969) Studies on the origin of milk fat. A further study of bovine serum lipoproteins and an estimation of their contribution to milk fat. *Biochemical Journal*, **113**, 629–633.

Bissell, M.J. and Hall, H.G. (1987) Form and function in the mammary gland: the role of extracellular matrix, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and L.W. Daniel), Plenum Press, New York, pp. 97–146.

Blackburn, D.G., Hobbs, A.A. and Rosen, J.M. (1982) Rat β-casein cDNA: sequence analysis and evolutionary comparisons. *Nucleic Acids Research*, **10**, 2295–2307.

Blackburn, D.G., Hayssen, V. and Murphy, C.L. (1989) The origins of lactation and the evolution

of milk: a review with new hypotheses. *Mammal Review*, **19**, 1–26.

Blass, E.M., and Teicher, M.H. (1980) 'Suckling'. *Science*, **210**, 15–22.

Blaxter, K.L. (1961) Lactation and the growth of the young, in *Milk: the Mammary Gland and its Secretion*, Vol. 2, (eds S.K. Kon and A.T. Cowie), Academic Press, New York, pp. 305–361.

Blaxter, K.L. (1971) The comparative biology of lactation, in *Lactation*, (ed I.R. Falconer), Butterworths, London, pp. 51–69.

Bonner, W.N. (1984) Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symposia of the Zoological Society of London*, **51**, 253–272.

Bounous G., Kingshavn, P.A.L., Taveroff, A. and Gold, P. (1988) Evolutionary traits in human milk protein. *Medical Hypotheses*, **27**, 133–140.

Brandan, E. and Fleischer, B. (1982) Orientation and role of nucleoside diphosphatase and 5'-nucleotidase in Golgi vesicles from rat liver. *Biochemistry*, **21**, 4640–4645.

Breach, R.A. and Dils, R.R. (1975) Fatty acyl specificity of glycerol-3-phosphate esterification in lactating rabbit mammary gland. *International Journal of Biochemistry*, **6**, 329–340.

Breach, R.A., Dils, R.R. and Watts, R. (1973) Milk triglcerides: the degree of non-random associations of fatty acids. *Journal of Dairy Research*, **40**, 273–287.

Bresslau, E. (1920) *The Mammary Apparatus of the Mammalia*. Methuen, London.

Brew, K. (1970) Lactose synthetase: evolutionary origins, structure and control. *Essays in Biochemistry*, **6**, 93–118.

Brew, K. (1972) The complete amino acid sequence of guinea pig α-lactalbumin. *European Journal of Biochemistry*, **27**, 341–353.

Brew, K. and Hill, R.L. (1975) Lactose biosynthesis. *Reviews of Physiology, Biochemistry and Pharmacology*, **72**, 105–158.

Brew, K., Vanaman, T.C. and Hill, R.L. (1968) The role of α-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. *Proceedings of the National Academy of Science, USA*, **59**, 491–497.

Brew, K., Castellino, F.J., Vanaman, T.C. and Hill, R.L. (1970) The complete amino acid sequence of bovine α-lactalbumin. *Journal of Biological Chemistry*, **245**, 4570–4582.

Brignon, G., Ribadeau-Dumas, B., Mercier, J.C. and Pelissier, J.P. (1977) Complete amino acid sequence of bovine α_{s2}-casein. *FEBS Letters*, **76**, 274–279.

Brodbeck, U. and Ebner, K.E. (1966) Resolution of a soluble lactose synthetase with two protein components and solubilization of microsomal lactose synthetase. *Journal of Biological Chemistry*, **241**, 762–764.

Brodbeck, U., Denton, W.L., Tanahashi, N. and Ebner, K.E. (1967) The isolation and identification of the B protein of lactose synthetase as α-lactalbumin. *Journal of Biological Chemistry*, **242**, 1391–1397.

Brody, S. and Nisbet, R. (1938) Growth and development. A comparison of the amounts and energetic efficiencies of milk production in rat and dairy cow. *Missouri Agricultural Experiment Station Research Bulletin*, **185**, 1–30.

Brody, S., Riggs, J., Kaufman, K. and Herring, V. (1938) Growth and development with special reference to domestic animals. Energy-metabolism levels during gestation, lactation and post-lactation rest. *Missouri Agricultural Experiment Station Research Bulletin*, **281**, 1–43.

Brooks, C.L. and Landt, M. (1984) Calcium-ion and calmodulin-dependent K-casein kinase in rat mammary acini. *Biochemical Journal*, **224**, 195–200.

Brown, R.C., Fish, W.W., Hudson, B.J. and Ebner, K.E. (1977) Isolation and characterization of rat α-lactalbumin: a glycoprotein. *Biochimica et Biophysica Acta*, **491**, 82–92.

Burditt, L.J., Parker, D., Craig, R.K., Getova, T. and Campbell, P.N. (1981) Differential expression of α-lactalbumin and casein genes during the onset of lactation in the guinea pig mammary gland. *Biochemical Journal*, **194**, 999–1006.

Bussmann, L.E., Ward, S. and Kuhn, N.J. (1984) Lactose and fatty acid synthesis in lactating rat mammary gland. Effects of starvation, re-feeding and administration of insulin, adrenaline, streptozotocin and 2-bromo-α-ergocryptine. *Biochemical Journal*, **219**, 173–180.

Butler, J.E. (1974) Immunoglobulins of the mammary secretions, in *Lactation: a Comprehensive Treatise*, Vol. 3, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 217–255.

Capasso, J.M., Keenan, T.W., Abeijon, C. and Hirschberg, C.B. (1989) Mechanism of phosphorylation in the lumen of the Golgi apparatus. Translocation of adenosine 5–triphosphate into Golgi vesicles from rat liver and mammary

gland. *Journal of Biological Chemistry*, **264**, 5233–5240.

Carling, D., Clarke, P.R., Zammit, V.A. and Hardie, D.G. (1989) Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. *European Journal of Biochemistry*, **186**, 129–136.

Carlson, D.M., Jourdian, G.W. and Roseman, S. (1973) The sialic acids. XIV. Synthesis of sialyllactose by a sialytransferase from rat mammary gland. *Journal of Biological Chemistry*, **248**, 5742–5750.

Carrick, D.T. and Kuhn, N.J. (1978) Diurnal variation and response to food withdrawal of lactose synthesis in lactating rats. *Biochemical Journal*, **174**, 319–325.

Cary, C.A. (1920) Amino acids of the blood as the precursors of milk proteins. *Journal of Biological Chemistry*, **43**, 477–489.

Casey, C.E., Howell, R.R., Lonnerdal, B., Moser, P.B., Picciano, M.F. and Rumball, S.V. (1985) Principles of trace element analysis and notes on some important elements, in *Human Lactation: Milk Components and Methodologies*, (eds R.G. Jensen and M.C. Neville), Plenum Press, New York, pp. 223–236.

Chadwick, A. (1977) Comparison of milk-like secretions found in non-mammals. *Symposia of the Zoological Society of London*, **41**, 341–358.

Chaiyabutr, N., Faulkner, A. and Peaker, M. (1980) The utilization of glucose for the synthesis of milk components in the fed and starved lactating goat *in vivo*. *Biochemical Journal*, **186**, 301–308.

Chalk, P.A. and Bailey, E. (1979) Changes in the yield, and carbohydrate, lipid and protein content of milk during lactation in the rat. *Journal of Developmental Physiology*, **1**, 61–79.

Chambon, P., Dierich, A., Gaub, M.P., Jakowlev, S., Jongstra, J., Krust, A., Lepennec, J.P., Oudet, P. and Reudelhuber, T. (1984) Promoter elements of genes coding for proteins and modulation of transcription by estrogens and progesterone. *Recent Progress in Hormone Research*, **40**, 1–42.

Chesney, R.W. (1988) Taurine: is it required for infant nutrition? *Journal of Nutrition*, **118**, 6–10.

Christensen, H.N. (1984) Organic ion transport during seven decades: the amino acids. *Biochimica et Biophysica Acta*, **779**, 255–269.

Clark, A.J., Bessos, H., Bishop, J.O., Brown, P., Harrison, S., Lathe, R., McClenaghan, M, Prowse, C., Simons, J.P., Whitelaw, C.B. and Wilmut, I. (1989) Expression of human antihemophilic factor IX in the milk of transgenic sheep. *Biotechnology*, **7**, 487–492.

Clegg, R.A. (1988) Regulation of fatty acid uptake and synthesis in mammary and adipose tissues: contrasting roles for cyclic AMP. *Current Topics in Cellular Regulation*, **29**, 77–128.

Clegg, R.A. and Mullaney, I. (1985) Acute changes in the cyclic AMP content of rat mammary acini *in vitro*. Influence of physiological and pharmacological agents. *Biochemical Journal*, **230**, 239–246.

Clegg, R.A. and Ottey, K.A. (1990) Cyclic AMP-dependent protein kinase in mammary tissue of the lactating rat. Activity ratio and responsiveness of the target enzymes acetyl-CoA carboxylase and glycogen phosphorylase to β-adrenergic stimulation. *Biochemical Journal*, **265**, 769–775.

Clegg, R.A., West, D.W. and Aitchinson, R.E.D. (1987) Protein phosphorylation in rat mammary acini and in cytosol preparations *in vitro*. *Biochemical Journal*, **241**, 447–454.

Cohen, J. and Massey, B. (1984) *Animal Reproduction*. Edward Arnold, London, p. 67.

Committee on Medical Aspects of Food Policy (1984) *Diet and Cardiovascular Disease*. HMSO, London.

Cooper, A.P. (1840) *On the Anatomy of the Breast*. Long, Orme, Green, Brown, Longman, London.

Cooper, S. and Grigor, M.R. (1980) Fatty acid specificities of microsomal acyltransferases esterifying positions -1 and -2 of acylglycerols in mammary glands from lactating rats. *Biochemical Journal*, **187**, 289–295.

Corps, A.N., Brown, K.D., Rees, L.H., Carr, J. and Prosser, C.G. (1988) The Insulin-Like Growth Factor I content in human milk increases between early and full lactation. *Journal of Clinical Endocrinology and Metabolism*, **67**, 25–29.

Cowie, A.T. (1969) Variations in the yield and composition of the milk during lactation in the rabbit and the galactopoietic effect of prolactin. *Journal of Endocrinology*, **44**, 437–450.

Cowie, A.T. and Tindal, J.S. (1971) *The Physiology of Lactation*. Edward Arnold, London.

Cowie, A.T., Forsyth, I.A. and Hart, I.C. (1980) *Hormonal Control of Lactation*. Springer–Verlag, Berlin.

Crabtree, B. and Newsholme, E.A. (1987) A systematic approach to describing and analysing metabolic control systems. *Trends in Biochemical Sciences*, **12**, 4–12.

Crabtree, B., Taylor, D.J., Coombs, J.E., Smith, R.A., Templer, S.P. and Smith, G.H. (1981) The activities and intracellular distributions of enzymes of carbohydrate, lipid and ketone-body metabolism in lactating mammary glands from ruminants and non-ruminants. *Biochemical Journal*, **196**, 747–756.

Cremin, F.M. and Power, P. (1985) Vitamins in bovine and human milks, in *Developments in Dairy Chemistry*, vol 3, (ed. P.F. Fox), Applied Science Publishers, London, pp. 337–405.

Cross, B.A. (1977) Comparative physiology of milk removal. *Symposia of the Zoological Society of London*, **41**, 193–210.

Cross, B.A., Findlay, A.L.R. (1969) Comparative and sensory aspects of milk ejection, in *Lactogenesis: the Initiation of Milk Secretion at Parturition*, (eds M. Reynolds and S.J. Folley), University of Pennsylvania Press, Philadelphia, pp. 245–252.

Daly, M. (1979) Why don't males lactate? *Journal of Theoretical Biology*, **78**, 325–345.

Daniel, C.W. and Silberstein, G.B. (1987) Postnatal development of the ruminant mammary gland, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 3–36.

Daniel, H., Vohwinkel, M. and Rehner, G. (1990) Effect of casein and β-casomorphines on gastrointestinal motility in rats. *Journal of Nutrition*, **120**, 252–257.

Darwin, C. (1872) *The Origin of Species*, 6th edn, John Murray, London.

Davies, D.T., Holt, C. and Christie, W.W. (1983) The composition of milk, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 71–117.

Davis, A.J., Fleet, I.R., Goode, J.A., Hamon, M.H., Maule Walker, F.M. and Peaker, M. (1979) Changes in mammary function at the onset of lactation in the goat: correlation with hormonal changes. *Journal of Physiology*, **288**, 33–44.

Davis, S.R. and Collier, R.J. (1985) Mammary blood flow and regulation of substrate supply for milk synthesis. *Journal of Dairy Science*, **68**, 1041–1058.

Davis, S.R. and Mepham, T.B. (1974) Amino acid and glucose uptake by the isolated guinea pig mammary gland. *Quarterly Journal of Experimental Physiology*, **59**, 113–130.

Dayal, R., Hurlimann, J., Suard, Y.M.L. and Kraehenbuhl, J.P. (1982) Chemical and immunochemical characterization of caseins and the major whey proteins of rabbit milk. *Biochemical Journal*, **201**, 71–79.

Delouis, C. (1975) Milk protein synthesis *in vitro*. Hormonal regulation. *Modern Problems in Paediatrics*, **15**, 16–30.

Dembinski, T.S. and Shiu, R.P.C. (1987) Growth factors in mammary gland development and function, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 355–381.

Dils, R.R. (1983) Milk fat synthesis, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 141–157.

Dugdale, A.E. (1986) Evolution and infant feeding. *Lancet*, **i**, 670–673.

Easter, D.J., Patton, S. and McCarthy, R.D. (1971) Metabolism of phospholipid in mammary gland. I. The supply of phospholipid for milk synthesis in the rat and goat. *Lipids*, **6**, 844–849.

Ebner, K.E. and Brodbeck, U. (1968) Biological role of α-lactalbumin: a review. *Journal of Dairy Science*, **51**, 317–322.

Ebner, K.E. and Schanbacher, F.L. (1974) Biochemistry of lactose and related carbohydrates in *Lactation: a Comprehensive Treatise*, Vol. 2, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 77–113.

Etindi, R.N. and Rillema, J.A. (1988) Prolactin induces the formation of inositol bisphosphate and inositol trisphosphate in cultured mouse mammary gland explants. *Biochimica et Biophysica Acta*, **968**, 385–391.

Farrell, H.M. (1973) Models for casein micelle formation. *Journal of Dairy Science*, **56**, 1195–1206.

Farrell, H.M. and Thompson, M.P. (1971) Biological significance of milk protein polymorphism. *Journal of Dairy Science*, **54**, 1219–1228.

Farrell, H.M., Leung, C.T. and Wickham, E.D. (1992) Distribution of ADPase activity in the lactating rat mammary gland and its possible role in an ATP cycle in the Golgi apparatus. *Archives of Biochemistry and Biophysics*, **292**, 368–375.

Faulkner, A. and Peaker, M. (1987) Regulation of mammary glucose metabolism in lactation in, *The Mammary Gland*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 536–562.

Faulkner, A., Chaiyabutr, N., Peaker, M., Carrick, D.T. and Kuhn, N.J. (1981) Metabolic significance of milk glucose. *Journal of Dairy Research*, **48**, 51–56.

Ferrier, B.M., Johns, T., Say, A. and Branda, L.A. (1973) Thiol–protein disulfide oxidoreductase activity in lactating and non–lactating mouse mammary gland. *Canadian Journal of Biochemistry*, **51**, 1555–1558.

Filer, L.J., Mattson, F.H. and Fomon, S.J. (1969) Triglyceride configuration and fat adsorption by the human infant. *Journal of Nutrition*, **99**, 293–298.

Findlay, J.B.C. and Brew, K. (1972) The complete amino acid sequence of human α-lactalbumin. *European Journal of Biochemistry*, **27**, 65–86.

Fitzgerald, D.K., Brodbeck, U., Kiyosawra, I., Mawral, R., Colvin, B. and Ebner, K.E. (1970) α-lactalbumin and the lactose synthetase reaction. *Journal of Biological Chemistry*, **245**, 2103–2108.

Fleet, I.R. and Mepham, T.B. (1983) Physiological methods used in the study of mammary substrate utilization in ruminants, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 469–500.

Fleet, I.R., Goode, J.A., Hamon, M.H., Laurie, M.S., Linzell, J.L. and Peaker, M. (1975) Secretory activity of goat mammary glands during pregnancy and the onset of lactation. *Journal of Physiology*, **251**, 763–773.

Fleet, I.R., Davis, A., Richardson, M. and Heap, R.B. (1992) The effect of recombinantly-derived insulin-like growth factor-I and insulin-like growth factor II on mammary vasodilation in the goat. *Journal of Physiology*, **446**, 69p.

Fleischmann, W. (1896) *The Book of the Dairy*. (Translated into English by C.M. Aikman and R.P. Wright), Blackie & Son, London.

Florkin, M. and Stotz, E.H. (eds) (1977) *Comprehensive Biochemistry*, Vol. 32, Elsevier, Amsterdam, p. 301.

Flynn, A. and Power, P. (1985) Nutritional aspects of minerals in bovine and human milks, in *Developments in Dairy Chemistry*, Vol. 3, (ed. P.F. Fox), Applied Science Publishers, London, pp. 183–215.

Folley, S.J. and French, T.H. (1949) The intermediary metabolism of the mammary gland. Respiration and acid production of mammary tissue during pregnancy, lactation and involution in the rat. *Biochemical Journal*, **45**, 270–276.

Folley, S.J. and Greenbaum, A.L. (1947) Changes in the arginase and alkaline phosphatase contents of the mammary gland and liver of the rat during pregnancy, lactation and mammary involution. *Biochemical Journal*, **41**, 261–269.

Fox, P.F. (ed.) (1982, 1983, 1985) *Developments in Dairy Chemistry*, Vols 1–3, Applied Science Publishers, London.

Franke, W.W., Lüder, M.R., Kartenbeck, J., Zerban, H. and Keenan, T.W. (1976) Involvement of vesicle coat material in casein secretion and surface regeneration. *Journal of Cellular Biology*, **69**, 173–195.

Fürstenburg, M.H.F. (1868) *Die Milchdrüsen der Kuh ihre Anatomy, Physiologie und Pathologie*. Engelmann, Leipzig.

Garel, J.P. (1974) Adjustment of tRNA population to match protein synthesis. *Journal of Theoretical Biology*, **43**, 211–225.

Garnsworthy, P.C. (ed.) (1988) *Nutrition and Lactation in the Dairy Cow*. Butterworths, London.

Gehring, U. (1987) Steroid hormone receptors: biochemistry, genetics and molecular biology. *Trends in Biochemical Science*, **12**, 399–402.

Geursen, A. and Grigor, M.R. (1987) Nutritional regulation of milk protein messenger RNA concentrations in mammary acini isolated from lactating rats. *Biochemistry International*, **15**, 873–879.

Gilbert, A.N. (1986) Mammary number and litter size in Rodentia: the 'one-half' rule. *Proceedings of the National Academy of Sciences, USA*, **83**, 4826–4830.

Girard, J., Burnol, A.F., Leturque, A., Ferré, P. (1987) Glucose homeostasis in pregnancy and lactation. *Biochemical Society Transactions*, **15**, 1028–1030.

Glock, G.E. and McLean, P. (1954) Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumours. *Biochemical Journal*, **56**, 171–175.

Godovac-Zimmerman, J. and Braunitzer, G. (1987) Modern aspects of the primary structure and function of β-lactoglobulins. *Milchwissenschaft*, **42**, 294–297.

Goldman, A.S., Atkinson, S.A. and Hanson, L.A. (eds) (1987) *Human Lactation 3: the Effects of Human Milk on the Recipient Infant*, Plenum Press, New York.

Gonzalez, R. and Verly, W.G. (1976) Isolation of an inhibitor of DNA synthesis specific for normal and malignant mammary cells. *Proceedings of the National Academy of Sciences, USA*, **73**, 2196–2220.

Gould, S.J. (1987) Freudian slip. *Natural History*, **2.87**, 14–21.

Gravert, H.O. (ed.) (1987) *Dairy Cattle Production*, World Animal Science C3, Elsevier, Amsterdam.

Graves, B.M. and Duvall, D. (1983) A role for aggregation pheromones in the evolution of mammal-like reptile lactation. *Annals of Nutrition*, **122**, 835–839.

Griffiths, M. (1983) Lactation in monotremata and speculations concerning the nature of lactation in cretaceous multituberculata. *Acta Palaeontologica Polonica*, **28**, 93–102.

Grigor, M.R. and Warren, S.M. (1980) Dietary regulation of mammary lipogenesis in lactating rats. *Biochemical Journal*, **188**, 61–65.

Grigor, M.R., Carrington, J.M., Arthur, P.G. and Hartmann, P.E. (1989) Lack of correlation between milk glucose concentrations and rates of milk production in the rat. *Journal of Dairy Research*, **56**, 37–43.

Grosclaude, F., Mahé, M.F. and Ribadeau-Dumas, B. (1973) Structure primaire de la caséine α_{s1}et de la caséine β bovines. Correctif. *European Journal of Biochemistry*, **40**, 323–324.

Grunnet, I. and Knudsen, J. (1979) Fatty acid synthesis in lactating goat mammary gland. Medium chain fatty acid synthesis. *European Journal of Biochemistry*, **95**, 497–502.

Grunnet, I. and Knudsen, J. (1983) Medium-chain fatty acid synthesis by goat mammary gland fatty acid synthetase. *Biochemical Journal*, **209**, 215–222.

Gumaa, K.A., Greenbaum, A.L. and McLean, P. (1971) The control of pathways of carbohydrate metabolism in mammary gland, in *Lactation*, (ed I.R. Falconer), Butterworths, London, pp. 197–238.

Gumaa, K.A., Greenbaum, A.L. and McLean, P. (1973) Adaptive changes in satellite systems related to lipogenesis in rat and sheep mammary gland and in adipose tissue. *European Journal of Biochemistry*, **34**, 188–198.

Gupta, P., Rosen, J.M., D'Eustachio, P. and Ruddle, F.H. (1982) Localization of the casein gene family to a single mouse chromosome. *Journal of Cell Biology*, **93**, 199–204.

Guyette, W.A., Matusik, R.J., Rosen, J.M. (1979) Prolactin-mediated transcriptional and post–transcriptional control of casein gene expression. *Cell*, **17**, 1013–1023.

György, P. (1953) A hitherto unrecognized biochemical difference between human milk and cow's milk. *Paediatrics*, **11**, 98–108.

Haldane, J.B.S. (1965) The possible evolution of lactation. *Zoologische Jahrbücher Systematique*, **92**, 41–48.

Hall, L., Laird, J.E., Pascall, J.C., Craig, R.K. (1984a) Guinea pig casein A cDNA. Nucleotide sequence analysis and comparison of the deduced protein sequence with that of bovine α_{s2}casein. *European Journal of Biochemistry*, **138**, 585–589.

Hall, L., Laird, J.E. and Craig, R.K. (1984b) Nucleotide sequence determination of guinea pig casein B mRNA reveals homology with bovine and rat α_{s1}caseins and conservation of the non-coding regions of the mRNA. *Biochemical Journal*, **222**, 561–570.

von Haller, A. (1778) *Elementa Physiologiae Corporis Humani*, Part 2, Book 28, Section 1, Vol. 7. Societas Typographica, Lausanne.

Hambraeus, L. (1982) Nutritional aspects of milk proteins, in *Developments in Dairy Chemistry*, Vol. 1, (ed. P.F. Fox), Applied Science Publishers, London, pp. 289–313.

Hamosh, M. and Goldman, A.S. (eds) (1986) *Human Lactation 2: Maternal and Environmental Factors*. Plenum Press, New York.

Hamosh, M., Freed, L.M., Jones, J.B., Berkow, S.E., Bitman, J., Mehta, N.R., Happ, B. and Hamosh, P. (1985) Enzymes in human milk, in *Human Lactation: Milk Components and Methodologies*, (eds R.J. Jensen and M.C. Neville), Plenum Press, New York, pp. 251–266.

Hansen, H.O., Jensen, S.S. and Knudsen, J. (1986) Absence of monoacylglycerol pathway for triacylglycerol synthesis in goat mammary gland. *Biochemical Journal*, **238**, 173–176.

Hanwell, A. and Linzell, J.L. (1972) A simple technique for measuring the rate of milk secretion in the rat. *Comparative Biochemistry and Physiology*, **43A**, 259–270.

Hardie, D.G., McCarthy, A.D. and Braddock, M. (1986) Mammalian fatty acid synthase: a chimeric protein which has evolved by gene fusion. *Biochemical Society Transactions*, **14**, 568–570.

Harzer, G. and Haschke, F. (1989) Micronutrients in human milk, in *Micronutrients in milk and milk-based products*, (ed. E. Renner), Elsevier Applied Science, London, pp. 125–237.

Hasan, H.R., White, D.A. and Mayer, R.J. (1982) Extensive destruction of newly synthesized casein in mammary explants in organ culture. *Biochemical Journal*, **202**, 133–138.

Hawke, J.C. and Taylor, M.W. (1983) Influence of nutritional factors on the yield, composition and physical properties of milk fat, in *Developments in Dairy Chemistry*, Vol. 2, (ed. P.F. Fox), Applied Science Publishers, New York, pp. 37–87.

Hayssen, V. and Blackburn, D.G. (1985) α-lactalbumin and the origins of lactation. *Evolution*, **39**, 1147–1149.

Haystead, T.A.J. and Hardie, D.G. (1986) Evidence that activation of acetyl-CoA carboxylase by insulin in adipocytes is mediated by a low M_r effector and not by increased phosphorylation. *Biochemical Journal*, **240**, 99–106.

Head, J.R. and Beer, A.E. (1978) The immunological role of viable leukocytic cells in mammary exosecretions, in *Lactation: a Comprehensive Treatise*, Vol. 4, (ed. B. Larson), Academic Press, New York, pp. 337–364.

Heald, C.W. and Saake, R.G. (1972) Cytological comparison of milk protein synthesis of rat mammary tissue *in vivo* and *in vitro*. *Journal of Dairy Science*, **55**, 621–628.

Henderson, A.J., Blatchford, D.R. and Peaker, M. (1983) The effects of milking thrice instead of twice daily on milk secretion in the goat. *Quarterly Journal of Experimental Physiology*, **68**, 645–652.

Henderson, A.J., Blatchford, D.R. and Peaker, M. (1985) The effects of long-term thrice-daily milking on milk secretion in the goat: evidence for mammary growth. *Quarterly Journal of Experimental Physiology*, **70**, 557–565.

Hennighausen, L.J., Steudle, A. and Sippel, A.E. (1982a) Nucleotide sequence of cloned cDNA coding for mouse κ-casein. *European Journal of Biochemistry*, **126**, 569–572.

Hennighausen, L.J., Sippel, A.E., Hobbs, A.A. and Rosen, J.M. (1982b) Comparative sequence analysis of mRNAs coding for mouse and rat whey acidic protein. *Nucleic Acids Research*, **10**, 3733–3744.

Hernell, O. and Blackberg, L. (1985) Lipase and esterase activities in human milk, in *Human Lactation: Milk Components and Methodologies*, (eds R.J. Jensen and M.C. Neville), Plenum Press, New York, pp. 267–276.

Hillerton, J.E., Knight, C.H., Turvey, A., Wheatley, S.D. and Wilde, C.J. (1990) Milk yield and mammary function in dairy cows milked four times daily. *Journal of Dairy Research*, **57**, 285–294.

Hirose, M., Kato, T., Omori, K., Takeuchi, M., Yoshikawa, M., Sasaki, R. and Chiba, H. (1981) Purification and characterization of four components of rat caseins. *Biochimica et Biophysica Acta*, **671**, 139–145.

Hirsch, P.F., Baruch, H. and Chaikoff, I.L. (1954) The relation of glucose oxidation to lipogenesis in mammary tissue. *Journal of Biological Chemistry*, **210**, 785–797.

Hirschberg, C.D. and Snider, M.D. (1987) Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. *Annual Review of Biochemistry*, **56**, 63–87.

Hobbs, A.A. and Rosen, J.M. (1982) Sequence of rat α- and γ-casein mRNAs: evolutionary comparison of the calcium-dependent rat casein multigene family. *Nucleic Acids Research*, **10**, 8079–8098.

Hobbs, A.A., Richards, D.A., Kessler, D.J. and Rosen, J.M. (1982) Complex hormonal regulation of rat casein gene expression. *Journal of Biological Chemistry*, **257**, 3598–3605.

Hollman, K.H. (1959) L'ultrastructure de la glande mammaire normale de la souris en lactation. *Journal of Ultrastructural Research*, **2**, 423–443.

Hollman, K.H. (1974) Cytology and fine structure of the mammary gland, in *Lactation: a Comprehensive Treatise*, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 3–95.

Holt, C. and Dalgleish, D.G. (1986) Electrophoretic and hydrodynamic properties of bovine casein micelles interpreted in terms of particles with an outer hairy layer. *Journal of Colloid and Interface Science*, **114**, 513–524.

Hopp, T.P. and Woods, K.R. (1979) Primary structure of rabbit α-lactalbumin. *Biochemistry*, **18**, 5182–5191.

Hopper, K.E. and McKenzie, H.A. (1974) Comparative studies of α-lactalbumin and lysozyme: Echidna lysozyme. *Molecular Cell Biochemistry*, **3**, 93–108.

Horisberger, M. and Vonlanthen, M. (1980) Localization of glycosylated κ-casein in bovine casein micelles by lectin- labelled gold granules. *Journal of Dairy Research*, **47**, 185–191.

Houdebine, L.M. (1976) Effects of prolactin and progesterone on suppression of casein genes. *European Journal of Biochemistry*, **68**, 219–225.

Huxtable, R.J. and Lippincott, S.E. (1983) Relative contributions of the mother, the nurse and endogenous synthesis to the taurine content of the newborn and suckling rat. *Annals of Nutrition and Metabolism*, **27**, 107–116.

Janolino, V.G. and Swaisgood, H.E. (1975) Isolation and characterization of sulfhydryl oxidase from bovine milk. *Journal of Biological Chemistry*, **250**, 2532–2538.

Jelliffe, D.B. and Jelliffe, E.F.P. (1978) *Human Milk in the Modern World*, Oxford University Press, Oxford.

Jenness, R. (1974) The composition of milk, in *Lactation: a Comprehensive Treatise*, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 3–107.

Jenness, R. (1979) Comparative aspects of milk proteins. *Journal of Dairy Research*, **46**, 197–210.

Jenness, R. (1985) Biochemical and nutritional aspects of milk and colostrum, in *Lactation*, (ed. B.L. Larson), Iowa State University Press, Ames, pp. 166–197.

Jenness, R. (1986) Lactational performance of various mammalian species. *Journal of Dairy Science*, **69**, 869–885.

Jensen, R.G. and Neville, M.C. (eds) (1985) *Human Lactation: Milk Components and Methodologies*, Plenum Press, New York.

Jois, M., Annison, E.F., Gouden, J.M. and McDowell, G.H. (1984) Amino acid exchange in muscle and mammary tissue of cows. *Proceedings of the Nutritional Society of Australia*, **9**, 162.

Jollès, J., Fiat, A.M., Schoentgen, F., Alais, C. and Jollès, P. (1974) The amino acid sequence of sheep κ_A-casein. Sequence studies concerning the κ_A-caseinoglycopeptide and establishment of the complete primary structure of the protein. *Biochimica et Biophysica Acta*, **365**, 335–343.

Jones, W.K., Yu-Lee, L., Clift, S.M., Brown, T.L. and Rosen, J.M. (1985) The rat casein multigene family. Fine structure and evolution of the β-casein gene. *Journal of Biological Chemistry*, **260**, 7042–7050.

Kabara, J.J. (1980) Lipids as host-resistance factors in human milk. *Nutritional Reviews*, **38**, 65–73.

Kacser, H. and Porteous, J.W. (1987) Control of metabolism: what do we have to measure? *Trends in Biochemical Sciences*, **12**, 5–13.

Kamerling, J.P., Dorland, L., van Halbeek, H., Vliegenthart, J.F.G., Messer, M. and Schauer, R. (1982) Structural studies of 4-O-acetyl-α-N-acetylneuraminyl-(2→3) lactose, the main oligosaccharide in echidna milk. *Carbohydrate Research*, **100**, 331–340.

Katz, J. and Wals, P.A. (1972) Pentose cycle and reducing equivalents in rat mammary gland slices. *Biochemical Journal*, **128**, 879–899.

Keenan, T.W., Morré, D.J. and Cheetham, R.D. (1970) Lactose synthesis by a Golgi apparatus fraction from rat mammary gland. *Nature (London)*, **228**, 1105–1106.

Kemp, T.S. (1982) *Mammal-like Reptiles and the Origin of Mammals*, Academic Press, London.

Keuper, H.J.K., Klein, R.A. and Spenner, F. (1985) Spectroscopic investigations on the binding site of bovine hepatic fatty acid biding protein. Evidence for the existence of a single binding site for two fatty acid molecules. *Chemistry and Physics of Lipids*, **38**, 159–173.

Kiermeier, F. and Petz, E. (1967) Über ein sulfhydrylgruppenoxydierendes Enzym in der Milch. Isolierung und Charakterisierung des Enzyms. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, **132**, 342–352.

Kinkade, J.M., Miller, W.W.K. and Segars, F.M. (1976) Isolation and characterization of murine lactoferrin. *Biochimica et Biophysica Acta*, **446**, 407–418.

Kinsella, J.E. (1968) The incorporation of [^{14}C3]-glycerol into lipids by dispersed bovine mammary cells. *Biochimica et Biophysica Acta*, **164**, 540–549.

Kinsella, J.E. (1972) Stearoyl-CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine. *Lipids*, **7**, 349–355.

Kinsella, J.E. (1976) Monoacyl-sn-glycerol 3 phosphate acyltransferase specificity in bovine mammary microsomes. *Lipids*, **11**, 680–684.

Kitchen, B.J. (1985) Indigenous milk enzymes, in *Developments in Dairy Chemistry*, Vol. 3, (ed. P.F. Fox), Applied Science Publishers, London, pp. 239–279.

Klee, W.A. and Klee, C.B. (1970) The role of α-lactalbumin in lactose synthetase. *Biochemical and Biophysical Research Communications*, **39**, 833–841.

Knight, C.H. (1984) Mammary growth and development: strategies of animals and investigators. *Symposia of the Zoological Society of London*, **51**, 147–170.

Knight, C.H. (1987) Compensatory changes in mammary development and function after hemimastectomy in lactating goats. *Journal of Reproduction and Fertility*, **79**, 343–352.

Knight, C.H., Doherty, A.H. and Peaker, M. (1984) Milk yield in rats in relation to activity and size of the mammary secretory cell population. *Journal of Dairy Research*, **51**, 29–35.

Knight, C.W., Wilde, C.J. and Peaker, M. (1988) Manipulation of milk secretion, in *Nutrition and*

Lactation in the Dairy Cow, (ed P.C. Garnsworthy), Butterworths, London, pp. 3–14.

Knudsen, J. (1979) Medium-chain fatty acid synthesis in lactating rabbit mammary gland. Intracellular concentration and specificity of medium-chain acyl thioester hydrolase. *Biochemical Journal*, **181**, 267–274.

Koldovsky, O. (1989) Search for role of milk-born biologically active peptides for the suckling. *Journal of Nutrition*, **119**, 1543–1551.

Kronfeld, D.S. (1987) The challenge of BST. *Large Animal Veterinarian*, **Nov/Dec 1987**, 14–17.

Kuhn, N.J. (1968) Lactogenesis in the rat. Metabolism of uridine diphosphate galactose by mammary gland. *Biochemical Journal*, **106**, 743–748.

Kuhn, N.J. (1972) The lactose and neuraminlactose content of rat milk and mammary tissue. *Biochemical Journal*, **130**, 177–180.

Kuhn, N.J. (1977) Lactogenesis: the search for trigger mechanisms in different species. *Symposia of the Zoological Society of London*, **41**, 165–192.

Kuhn, N.J. (1978) Glucose as a fuel for the mammary gland. *Biochemical Society Transactions*, **6**, 539–543.

Kuhn, N.J. (1983) The biochemistry of lactogenesis, in *Biochemistry of Lactation*, (ed T.B. Mepham), Elsevier, Amsterdam, pp. 351–379.

Kuhn, N.J. and Fisher, C. (1989) Nutritional and mild stress effects on mammary lactose and fatty acid synthesis in intact lactating mice. *Biochemical Society Transactions*, **17**, 777–778.

Kuhn, N.J. and Lowenstein, J.M. (1967) Lactogenesis in the rat. Changes in metabolic parameters at parturition. *Biochemical Journal*, **105**, 995–1002.

Kuhn, N.J. and White, A. (1975a) The topography of lactose synthetase. *Biochemical Journal*, **148**, 77–84.

Kuhn, N.J. and White, A. (1975b) Milk glucose as an index of the intracellular glucose concentration of rat mammary gland. *Biochemical Journal*, **152**, 153–155.

Kuhn, N.J. and White, A. (1977) The role of nucleoside diphosphatase in a uridine nucleotide cycle associated with lactose synthesis in rat mammary gland Golgi apparatus. *Biochemical Journal*, **168**, 423–433.

Kuhn, N.J., Wooding, F.B.P. and White, A. (1980) Properties of galactosyltransferase–enriched vesicles of Golgi membranes from lactating-rat mammary gland. *European Journal of Biochemistry*, **103**, 377–385.

Kuhn, N.J., Ward, S. and Leong, W.S. (1991) Submicromolar manganese dependence of Golgi vesicular galactosyltransferase (lactose synthetase). *European Journal of Biochemistry*, **195**, 243–250.

Kulski, J.K., Smith, M. and Hartmann, P.E. (1977) Perinatal concentrations of progesterone, lactose and α-lactalbumin in the mammary secretion of women. *Journal of Endocrinology*, **74**, 509–510.

Kulski, J.K., Topper, Y.J., Chomezysnki, P. and Qasba, P. (1983a) An essential role for glucocorticoid in casein gene expression in rat mammary explants. *Biochemical and Biophysical Research Communications*, **114**, 380–387.

Kulski, J.K., Nicholas K.R., Topper, Y.J. and Qasba, P. (1983b) Essentiality of insulin and prolactin for accumulation of rat casein mRNAs. *Biochemical and Biophysical Research Communications*, **116**, 994–999.

Larson, B.L. (ed.) (1985) *Lactation*. Iowa State University Press, Ames.

Larson B.L. and Smith, V.R. (eds) (1974–78) *Lactation*, Vols 1–4, Academic Press, New York.

Lascelles, A.K. (1977) Role of the mammary gland and milk in immunology. *Symposia of the Zoological Society of London*, **41**, 241–260.

Lascelles, A.K. and Lee, C.S. (1978) Involution of the mammary gland, in *Lactation: a Comprehensive Treatise*, Vol. 4, (ed. B.L. Larson), Academic Press, New York, pp. 115–177.

Latham, M.C. (1986) Evolution and infant feeding. *Lancet*, **i**, p. 1089.

Leong, W.S. (1988) Synthesis of sialyllactose by the lactating-rat mammary gland. *Doctoral Thesis*, University of Birmingham.

Leong, W.S., Leedham, S.A. and Kuhn, N.J. (1987) Cationic activation of galactosyltransferase and sialyltransferase from a Golgi-enriched fraction of rat mammary gland. *Biochemical Society Transactions*, **15**, 1079–1080.

Leong, W.S., Navaratnam, N., Stankiewicz, M.J., Wallace, A.V., Ward, S. and Kuhn, N.J. (1990) Subcellular compartmentation in the synthesis of the milk sugars lactose and α-2,3 sialyllactose. *Protoplasma*, **159**, 144–156.

Lillegraven, J.A. (1980) Introduction, in *Mesozoic Mammals*, (eds J.A. Lillegraven, Z. Kielen-Jaworowska and W.A. Clemens), University of California Press, Berkeley, pp. 1–6.

Lin, C.Y. and Kumar, S. (1971) Primer specificity of mammary fatty acid synthetase and the role

of the soluble β-oxidative enzymes. *Journal of Biological Chemistry*, **246**, 3284–3290.

Lingappa, V.R., Lingappa, J.R., Prasad, R., Ebner, K.E. and Blobel, G. (1978) Coupled cell-free synthesis, segregation, and core glycosylation of a secretory protein. *Proceedings of the National Academy of Sciences, USA*, **75**, 2338–2342.

Linzell, J.L. (1959) Physiology of the mammary glands. *Physiological Reviews*, **39**, 534–576.

Linzell, J.L. (1966) Measurement of venous flow by continuous thermodilution and its application to measurement of mammary blood flow in the goat. *Circulation Research*, **18**, 745–754.

Linzell, J.L. (1974) Mammary blood flow and methods of identifying and measuring precursors of milk, in *Lactation: a Comprehensive Treatise*, vol 1, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 143–225.

Linzell, J.L. and Peaker, M. (1971) Mechanism of milk secretion. *Physiological Reviews*, **51**, 564–597.

Linzell, J.L. and Peaker, M. (1974) Changes in colostrum composition and in the permeability of the mammary epithelium at about the time of parturition in the goat. *Journal of Physiology (London)*, **243**, 129–151.

Linzell, J.L., Mepham, T.B., Annison, E.F. and West, C.E. (1969) Mammary metabolism in lactating sows: arteriovenous differences of milk precursors and the mammary metabolism of [^{14}C] and [^{14}C] acetate. *British Journal of Nutrition*, **23**, 319–332.

Linzell, J.L., Mepham, T.B. and Peaker, M. (1976) The secretion of citrate into milk. *Journal of Physiology (London)*, **260**, 739–750.

Lobato, M.F., Ros, M., Moreno, F.J. and Garcia-Ruiz, J.P. (1986) Nutritional and hormonal regulation of malic enzyme synthesis in rat mammary gland. *Biochemical Journal*, **236**, 441–445.

Loizzi, R.F. (1987) Mammary cytoskeleton and the regulation of microtubules, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 147–179.

Long, C.A. (1972) Two hypotheses on the origin of lactation. *American Naturalist*, **106**, 141–144.

Lonnerdal, B. (1985) Methods for studying trace-element binding ligands in human milk, in *Human Lactation: Milk Components and Methodologies*, (eds R.J. Jensen and M.C. Neville), Plenum Press, New York, pp. 243–248.

Loudon, A.S.I. and Racey, P.A. (eds) (1987) *Reproductive Energetics in Mammals*, Clarendon Press, Oxford.

McArthur, R.H. and Wilson, E.O. (1967) *The Theory of Island Biogeography*, Princeton University Press, Princeton.

McBride, O.W. and Korn, E.D. (1963) The lipoprotein lipase of mammary gland and the correlation of its activity to lactation. *Journal of Lipid Research*, **4**, 17–20.

McDougall, E.I., Stewart, J.C. (1976) The whey proteins of the milk of Red Deer (*Cervus elaphus L*). A homologue of bovine β-lactoglobulin. *Biochemical Journal*, **153**, 647–655.

MacGillivray, R.T.A., Brew, K. and Barnes, K. (1979) The amino acid sequence of goat α-lactalbumin. *Archives of Biochemistry and Biophysics*, **197**, 404–414.

Mackall, J.C. and Lane, M.D. (1976) Changes in mammary-gland acetyl-coenzyme A carboxylase associated with lactogenic differentiation. *Biochemical Journal*, **162**, 635–642.

McKenzie, L., Fitzgerald, D.K. and Ebner, K.E. (1971) Lactose synthetase activities in rat and mouse mammary glands. *Biochimica et Biophysica Acta*, **230**, 526–530.

McKenzie, R.M. and Larson, B.L. (1978) Purification and partial characterization of a unique group of phosphoproteins from rat milk whey. *Journal of Dairy Science*, **61**, 723–728.

McLean, P. (1958a) Carbohydrate metabolism of mammary tissue. Pathways of glucose catabolism in the mammary gland. *Biochimica et Biophysica Acta*, **30**, 303–315.

McLean, P. (1958b) Carbohydrate metabolism of mammary tissue. Levels of oxidised and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide in the rat mammary gland. *Biochimica et Biophysica Acta*, **30**, 316–324.

McNeillie, E.M., Clegg, R.A. and Zammit, V.A. (1981) Regulation of acetyl-CoA carboxylase in rat mammary gland. Effects of incubation with Ca, Mg and ATP on enzyme activity in tissue extracts. *Biochemical Journal*, **200**, 639–644.

McTiernan, A. and Thomas, D.B. (1986) Evidence for a protective effect of lactation on risk of breast cancer in young women. *American Journal of Epidemiology*, **124**, 353–358.

Madon, R.J., Martin, S., Davies, A. Fawcett, H.A.C., Flint, D.J. and Baldwin, S.A. (1990) Identification and characterization of glucose transport proteins in plasma membrane and Golgi-vesicle enriched fractions prepared from lactating rat mammary gland. *Biochemical Journal*, **272**, 307–312.

Marshall, M.O. and Knudsen, J. (1977) The specificity of 1-acyl-sn-glycerol 3 phosphate acyltransferase in microsomal fractions from lactating cow mammary gland towards short, medium and long chain acyl-CoA esters. *Biochimica et Biophysica Acta*, **489**, 236–241.

Marshall, M.O. and Knudsen, J. (1979) Specificity of diacylglycerol acyltransferase from bovine mammary gland, liver and adipose tissue towards acyl-CoA esters. *European Journal of Biochemistry*, **94**, 93–98.

Martin, R.D. (1984) Scaling effects and adaptive strategies in mammalian lactation. *Symposia of the Zoological Society of London*, **51**, 87–117.

Martin, R.H., Glass, M.R., Chapman, C., Wilson, G.D. and Woods, K.L. (1980) Human α-lactalbumin and hormonal factors in pregnancy and lactation. *Clinical Endocrinology*, **13**, 223–230.

Martin, R.H., Glass, M.R., Chapman, C., Wilson, G.D. and Woods, K.L. (1981) Suppression by bromocriptine of the serum lactalbumin peak associated with lactogenesis. *Clinical Endocrinology*, **14**, 363–366.

Martyn, P. and Hansen, I.A. (1980) Initiation of fatty acid synthesis in rat mammary glands. *Biochemical Journal*, **190**, 171–175.

Martyn, P. and Hansen, I.A. (1981) Initiation of lipogenic enzyme activities in rat mammary glands. *Biochemical Journal*, **198**, 187–192.

Mather, I.H. (1987) Proteins of the milk–fat–globule membrane as markers of mammary epithelial cells and apical plasma membrane, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 217–267.

Mather, I.H. and Keenan, T.W. (1983) Function of endomembranes and the cell surface in the secretion of organic milk constituents, in *Biochemistry of Lactation*, (ed T.B. Mepham), Elsevier, Amsterdam, pp. 231–283.

Matyukov, V.S. and Urynshev, A.P. (1980) Linkage between cattle milk α_{s1}-, β-, κ-casein loci. *Genetika*, **16**, 884–886.

Maule Walker, F.M. (1984) Exocrine and endocrine secretions of the mammary glands; local control in ruminants. *Symposia of the Zoological Society of London*, **51**, 171–191.

Maule Walker, F.M. and Peaker, M. (1980) Local production of prostaglandins in relation to mammary function at the onset of lactation in the goat. *Journal of Physiology (London)*, **309**, 65–79.

Mayer, R.J. (1978) Hormonal factors in lipogenesis in mammary gland. *Vitamins and Hormones*, **36**, 101–163.

Mayer, R.J., Burgess, R.J. and Russell, S.M. (1980) Factors controlling intracellular breakdown of proteins, in *Protein Deposition in Animals*, (eds P.J. Buttery and D.B. Lindsay), Butterworths, London, pp. 21–49.

Medawar, P.B. and Medawar, J.S. (1984) *Aristotle to Zoos: a Philosophical Dictionary of Biology*, Weidenfeld & Nicolson, London, pp. 41–42.

Mendelson, C.R., Zinder, O., Blanchette-Mackie, E.J., Chernick, S.S. and Scow, R.O. (1977) Lipoprotein lipase and lipid metabolism in mammary gland. *Journal of Dairy Science*, **60**, 666–676.

Mepham, T.B. (1982) Amino acid utilization by lactating mammary gland. *Journal of Dairy Science*, **65**, 287–298.

Mepham, T.B. (ed.) (1983) *Biochemistry of Lactation*, Elsevier, Amsterdam.

Mepham, T.B. (1986) The development of ideas on milk protein secretion, *Vlaams Diergeneeskundig Tijdschrift*, **55**, 228–238.

Mepham, T.B. (1987a) *Physiology of Lactation*, Open University Press, Milton Keynes.

Mepham, T.B. (1987b) Changing prospects and perspectives in dairy research. *Outlook on Agriculture*, **16**, 182–188.

Mepham, T.B. (1988) Nutrient uptake by the lactating mammary gland, in *Nutrition and Lactation in the Dairy Cow*, (ed. P.C. Garnsworthy), Butterworths, London, pp. 15–31.

Mepham, T.B. (1989a) Science and the politics of breastfeeding. *Science and Public Policy*, **16**, 181–191.

Mepham, T.B. (1989b) Criteria for the public acceptability of biotechnological innovations in animal production, in *Biotechnology in Growth Regulation*, (eds R.B. Heap, C.G. Prosser and G.E. Lamming), Butterworths, London, pp. 203–212.

Mepham, T.B. (1989c) Questions of validity in mammary physiology: methodology and ethics. *Proceedings of the Nutrition Society*, **48**, 1–7.

Mepham, T.B. (1991) Control of milk production, in *Biotechnology in Livestock in Developing Countries*, (ed A.G. Hunter), Centre for Tropical Veterinary Medicine, Edinburgh, pp. 186–200.

Mepham, T.B. (1992) Public health implications of bovine somatotrophin use in dairying: discussion paper. *Journal of the Royal Society of Medicine*, **85**, 736–739.

Mepham, T.B. (1993) The development of ideas on

the role of glucose in regulating milk secretion. *Australian Journal of Agricultural Research*, **44**, 509–522.

Mepham, T.B., Overthrow, J., Short, A.H. and Warne, D.E. (1984) Amino acid transport interactions in lactating mammary gland of guinea pig. *Journal of Physiology (London)*, **257**, 73p.

Mepham, T.B. and Linzell, J.L. (1967) Urea formation by the lactating goat mammary gland. *Nature* (London), **214**, 507–508.

Mepham, T.B., Gaye, P., Martin, P. and Mercier, J.C. (1992) Biosynthesis of milk protein, in *Advanced Dairy Chemistry*, Vol. 1, *Proteins*, (ed. P.F. Fox), Elsevier, London and New York, pp. 491–543.

Mercer, S.W. and Williamson, D.H. (1987) The regulation of lipogenesis *in vivo* in the lactating mammary gland of the rat during the starved–refed transition. Studies with acarbose, a glucosidase inhibitor. *Biochemical Journal*, **242**, 235–243.

Mercier, J-C, and Gaye, P. (1983) Milk protein synthesis, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 177–227.

Messer, M. (1974) Indentification of N-acetyl-4-O-acetylneuraminyl-lactose in Echidna milk. *Biochemical Journal*, **139**, 415–420.

Messer, M., Trikonoff, E., Stern, W., Collins, J.G. and Bradbury, J.H. (1980) Structure of a marsupial-milk trisaccharide. *Carbohydrate Research*, **83**, 327–334.

Messer, M., Gadriel, P.A., Ralston, G.B. and Griffiths, M. (1983) Carbohydrates of the milk of the platypus. *Australian Journal of Biological Sciences*, **36**, 129–137.

Metz-Boutigue, M.H., Jollès, J., Mazurier, J., Schoentgen, F., Legrand, D., Spik, G., Montreuil, J. and Jollès, P. (1984) Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *European Journal of Biochemistry*, **145**, 659–676.

Mikkelsen, J., Hojrup, P., Hensen, H.J., Hansen, J.K. and Knudsen, J. (1985) Evidence that the medium-chain acyltransferase of lactating-goat mammary gland fatty acid synthetase is identical with the acetyl/malonyltransferase. *Biochemical Journal*, **227**, 981–985.

Milstein, C., Brownlee, G.G., Harrison, T.M. and Matthews, M.B. (1972) A possible precursor of immunoglobulin light chains. *Nature New Biology (London)*, **239**, 117–120.

Mitani, M., Harushima, Y., Kuwashima, K., Ikeguchi, M. and Sugai, S. (1986) Innocuous character of [ethylenebis(oxyethylenenitrilo)] tetraacetic acid and EDTA as metal-ion buffers in studying Ca binding by α-lactalbumin. *Journal of Biological Chemistry*, **261**, 8824–8829.

Mix, L.S. (1987) Potential impact of the growth hormone and other technology on the United States Dairy Industry by the year 2000. *Journal of Dairy Science*, **70**, 487–497.

Montreuil, J. (1980) Primary structure of glycoprotein glycans. Basis for the molecular biology of glycoproteins. *Advances in Carbohydrate Chemistry and Biochemistry*, **37**, 157–223.

Moore, A., Boulton, A.P., Heid, H.W., Jarasch, E.D. and Craig, R.K. (1985) Purification and tissue-specific expression of casein kinase from the lactating guinea pig mammary gland. *European Journal of Biochemistry*, **152**, 729–737.

Morgan, G. and Wooding, F.B.P. (1982) A freeze-fracture study of tight junction structure in sheep mammary gland epithelium during pregnancy and lactation. *Journal of Dairy Research*, **49**, 1–11.

Morgensen, I.B., Schulenberg, H., Hansen, H.O., Spener, F. and Knudsen, J. (1987) A novel acyl-CoA-binding protein from bovine liver. Effect on fatty acid synthesis. *Biochemical Journal*, **241**, 189–192.

Morriss, F.H. (1985) Methods for investigating the presence and physiologic role of growth factors in milk, in *Human Lactation: Milk Components and Methodologies*, (eds R.G. Jensen and M.C. Neville), Plenum Press, New York, pp. 193–200.

Munday, M.R. and Hardie, D.G. (1984) Isolation of three cyclic AMP-independent acetyl-CoA caboxylase kinases from lactating rat mammary gland, and characterization of their effects on enzyme activity. *European Journal of Biochemistry*, **141**, 617–627.

Munday, M.R. and Hardie, D.G. (1986) The role of acetyl-CoA carboxylase phosphorylation in the control of mammary gland fatty acid synthesis during the starvation and re-feeding of lactating rats. *Biochemical Journal*, **237**, 85–91.

Munday, M.R. and Hardie, D.G. (1987) Role of protein phosphorylation in the regulation of fatty acid synthesis in the mammary gland of the lactating rat, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 563–613.

Munday, M.R. and Williamson, D.H. (1983) Diurnal variations in food intake and in lipoge-

nesis in mammary gland and liver of lactating rats. *Biochemical Journal*, **214**, 183–187.

Murakami, K. and Berliner, L.J. (1983) A distinct zinc binding site in the α-lactalbumins regulates calcium binding. Is there a physiological role for this control? *Biochemistry*, **22**, 3370–3374.

Murakami, K., Andree, P.J. and Berliner, L.J. (1982) Metal ion binding to α-lactalbumin species. *Biochemistry*, **21**, 5488–5494.

Musci, G. and Berliner, L.J. (1985) Physiological roles of zinc and calcium binding to α-lactalbumin in lactose biosynthesis. *Biochemistry*, **24**, 6945–6948.

Naccarato, W.F., Ray, R.E. and Wells, W.W. (1975) Characterization and tissue distribution of 6-O-D-galactopyranosyl myo-inositol in the rat. *Journal of Biological Chemistry*, **250**, 1872–1876.

Nagamatsu, Y. and Oka, T. (1983) The differential actions of cortisol on the synthesis and turnover of α-lactalbumin and casein and on accumulation of their mRNA in mouse mammary gland in organ culture. *Biochemical Journal*, **212**, 507–515.

Nagato, T., Yoshida, H., Yoshida, A. and Uehara, U. (1980) A scanning electron microscope study of myoepithelial cells in exocrine glands. *Cell and Tissue Research*, **209**, 1–10.

Naggart, J., Williams, B., Cashman, D.P. and Smith, S. (1987) Cloning and sequencing of the medium-chain S-acyl fatty acid synthetase thioester hydrolase cDNA from rat mammary gland. *Biochemical Journal*, **243**, 597–601.

Nakhasi, H.L. and Qasba, P.K. (1979) Quantitation of milk proteins and their mRNAs in rat mammary gland in various stages of gestation and lactation. *Journal of Biological Chemistry*, **254**, 6016–6025.

Nandedkar, A.K.N. and Kumar, S. (1969) Biosynthesis of fatty acids in mammary tissue. Synthesis of butyrate in lactating rabbit mammary supernatant fraction by the reversal of β-oxidation. *Archives of Biochemistry and Biophysics*, **134**, 563–571.

Navaratnam, N., Virk, S.S., Ward, S. and Kuhn, N.J. (1986) Cationic activation of galactosyltransferase from rat mammary Golgi membranes by polyamines and by basic proteins and peptides. *Biochemical Journal*, **239**, 423–433.

Navaratnam, N., Ward, S., Fisher, C., Kuhn, N.J., Keen, J.N. and Findlay, J.B.C. (1988) Purification, properties and cation activation of galactosyltransferase from lactating-rat mammary Golgi membranes. *European Journal of Biochemistry*, **171**, 623–629.

Nelson, W.L., Kaye, A., Moore, M., Williams, H.H. and Herrington, B.L. (1951) Milking techniques and the composition of guinea pig milk. *Journal of Nutrition*, **44**, 585–594.

Neville, M.C. and Daniel, C.W. (eds) (1987) *The Mammary Gland: Development, Regulation and Function*, Plenum Press, New York.

Neville, M.C., Neifert, M.R. (eds) (1983) *Lactation: Physiology, Nutrition and Breastfeeding*, Plenum Press, New York.

Neville, M.C. and Watters, C. (1983) Secretion of calcium into milk: review. *Journal of Dairy Science*, **66**, 371–380.

Neville, M.C., Lobitz, C.J., Ripoll, E.A. and Tinney, C. (1980) The sites of aminoisobutyric acid uptake in normal mammary gland and ascites tumor cells. *Journal of Biological Chemistry*, **255**, 7311–7316.

Neville, M.C., Allen, J.C. and Casey, C.E. (1986) Regulation of the rate of lactose secretion, in *Human lactation 2. Maternal and Environmental Factors Affecting Human Lactation*, (eds M. Hamosh and A.S. Goldman), Plenum, New York, pp. 141–154.

Neville, M.C., Hay, W.W., Fennessey, P. (1990) Physiological significance of the concentration of human milk glucose. *Protoplasma*, **159**, 118–128.

Nicholas, K.R., Messer, M., Elliott, C., Maher, F. and Shaw, D.C. (1987) A novel whey protein synthesized only in late lactation by the mammary gland from the tammar (*Macropus eugenii*). *Biochemical Journal*, **241**, 899–904.

Nichols, B.L., McKee, K.S., Henry, J.F. and Putman, M. (1987) Human lactoferrin stimulates thymidine incorporation into DNA of rat crypt cells. *Pediatric Research*, **21**, 563–567.

Nicoll, M.E. and Thompson, S.D. (1987) Basal metabolic rates and energetics of reproduction in therian mammals: marsupials and placentals compared. *Symposia of the Zoological Society of London*, **57**, 7–27.

Oddy, V.H., Lindsay, D.B. and Fleet, I.R. (1988) Protein synthesis and degradation in the mammary gland of lactating goats. *Journal of Dairy Research*, **55**, 143–154.

Oftedal, O.T. (1984) Milk composition, milk yield and energy output at peak lactation: a comparative review. *Symposia of the Zoological Society of London*, **51**, 33–85.

O'Keefe, E.T., Hill, R.L. and Bell, J.E. (1980)

Active site of bovine galactosyltransferase: kinetic and fluorescence studies. *Biochemistry*, **19**, 4954–4962.

Ottey, K.A., Takhar, S. and Munday, M.R. (1989) Comparison of two cyclic-nucleotide-independent acetyl-CoA carboxylase kinases from lactating rat mammary gland; identification of the kinase responsible for acetyl-CoA carboxylase inactivation *in vivo*. *Biochemical Society Transactions*, **17**, 349–350.

Page, T. and Kuhn, N.J. (1986) Arteriovenous glucose differences across mammary gland of the fed, starved and re-fed lactating rat. *Biochemical Journal*, **239**, 269–274.

Page, T. and Kuhn, N.J. (1987) Intracellular glucose in the mammary gland of the lactating rat. *Biochemical Society Transactions*, **15**, 1095–1096.

Paige, D.M. and Davis, L.R. (1985) Nutritional significance of lactose, in *Developments in Dairy Chemistry*, Vol. 3, (ed P.F. Fox), Applied Science Publishers, London, pp. 111–132.

Palmiter, R.D. (1969) Hormonal induction and regulation of lactose synthetase in mouse mammary gland. *Biochemical Journal*, **113**, 409–417.

Palmquist, D.L., Davis, C.L., Brown, R.E. and Sachan, D.S. (1969) Availability and metabolism of various substrates in ruminants. Entry rate into the body and incorporation into milk fat of D(-)β-hydroxybutyrate. *Journal of Dairy Science*, **52**, 633–638.

Papiz, M.Z., Sawyer, L., Eliopoulos, E.E., North, A.C.T., Findlay, J.B.C., Sivaprasadarao, R., Jones, T.A., Newcomer, M.E. and Kraulis, P.J. (1986) The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature (London)*, **324**, 383–385.

Pascall, J.C., Boulton, A.P. and Craig, R.K. (1981) Characterization of a membrane-bound serine-specific casein kinase isolated from lactating guinea pig mammary gland. *European Journal of Biochemistry*, **119**, 91–99.

Patton, S. and Jensen, R.G. (1976) *Biomedical Aspects of Lactation*, Pergamon Press, Oxford.

Paulson, J.C., Rearick, J.J. and Hill, R.L. (1977) Enzymatic properties of β-D-galactoside α2→6 sialyltransferase from bovine colostrum. *Journal of Biological Chemistry*, **252**, 2363–2361.

Peaker, M. (1977) Mechanism of milk secretion: composition in relation to potential difference across the mammary epithelium. *Journal of Physiology (London)*, **270**, 489–505.

Peaker, M. (1978) Ion and water transport in the mammary gland, in *Lactation: a Comprehensive Treatise*, Vol. 4, (ed B.L. Larson), Academic Press, New York, pp. 437–462.

Peaker, M. (1983) Secretion of ions and water, in *Biochemistry of Lactation*, (ed. T.B. Mepham), Elsevier, Amsterdam, pp. 285–305.

Peaker, M. and Goode, J.A. (1978) The milk of the elephant seal mirounga Leonina: in particular the composition of the aqueous phase. *Journal of Zoology (London)*, **185**, 469–486.

Peaker, M., Vernon, R.G. and Knight, C.H. (1984) Physiological strategies in lactation. *Symposia of the Zoological Society of London, Number 51*, Academic Press, London.

Peeters, G.J., Houvenaghel, A., Roets, E., Massart-Leen, A.M., Verbeke, R., Dhont, G. and Verschooten, F. (1979) Electromagnetic blood flow recording and balance of nutrients in the udder of lactating cows. *Journal of Animal Science*, **48**, 1143–1153.

Perez, M. and Hirschberg, C.B. (1986) Transport of sugar nucleotides and adenosine 3′-phosphate-5′-phosphosulfate into vesicles derived from the Golgi apparatus. *Biochimica et Biophysica Acta*, **864**, 213–222.

Phillips, D.C., Acharya, K.R., Handoll, H.H.G. and Stuart, D.T. (1987) From lysozyme structure to enzyme engineering. *Biochemical Society Transactions*, **15**, 737–744.

Pianka, E.R. (1970) On r- and K- selection. *American Naturalist*, **104**, 592–599.

Pitelka, D.R. (1985) Teamwork in the community of mammary cells. *Hannah Research Report* **1985**, 63–74.

Pitelka, D.R. and Hamamoto, S.T. (1983) Ultrastructure of the mammary secretory cell, in *Biochemistry of Lactation*, (ed T.B. Mepham), Elsevier, Amsterdam, pp. 29–70.

Pitelka, D.R. and Taggart, B.W. (1983) Mechanical tension induces lateral movement of intramembrane components of the tight junction: studies on mouse mammary cells in culture. *Journal of Cell Biology*, **96**, 606–612.

Pond, C.M. (1976) The significance of lactation in the evolution of mammals. *Evolution*, **31**, 177–199.

Pond, C.M. (1983) Parental feeding as a determinant of ecological relationships in mesozoic terrestrial ecosystems. *Acta Palaeontologica Polonica*, **28**, 215–224.

Pond, C.M. (1984) Physiological and ecological importance of energy storage in the evolution of

lactation. *Symposia of the Zoological Society of London*, **51**, 1–32.

Powell, J.T. and Brew, K. (1976) Metal ion activation of galactosyltransferase. *Journal of Biological Chemistry*, **251**, 3645–3652.

Préaux, G., Braunitzer, G., Schrank, B. and Stangl, A. (1979) The amino acid sequence of goat β-lactoglobulin. *Hoppe Seyler Zeitschrift für Physiologische Chemie*, **360**, 1595–1604.

Prentice, A.M. and Whitehead, R.G. (1987) The energetics of human reproduction. *Symposia of the Zoological Society of London*, **57**, 275–304.

Prentice, A.M., Paul, A., Prentice, A., Black, A., Cole, T. and Whitehead, R. (1986) Cross–cultural differences in lactational performance, in *Human Lactation 2: Maternal and Environmental Factors*, (eds M. Hamosh and A.S. Goldman), Plenum Press, New York, pp. 13–44.

Prosser, C.G. and Topper, Y.J. (1986) Changes in the rate of carrier-mediated glucose transport by mouse mammary epithelial cells during ontogeny: hormone dependence delineated *in vitro*. *Endocrinology*, **119**, 91–96.

Qasba, P.K. and Safaya, S.K. (1984) Similarity of the nucleotide sequences of rat α-lactalbumin and chicken lysozyme genes. *Nature (London)*, **308**, 377–380.

Qasba, P.K., Hewlett, I.K. and Byers, S. (1983) The presence of the milk protein α-lactalbumin and its mRNA in the rat epididymis. *Biochemical and Biophysical Research Communications*, **117**, 306–312.

Ram, B.P. and Munjal, D.D. (1986) Galactosyltransferases: physical, chemical and biological aspects. *CRC Critical Reviews in Biochemistry*, **17**, 257–311.

Reiter, B. (1985a) The biological significance of non-immunoglobulin protective proteins in milk: lysozyme, lactoferrin, lactoperoxidase, in *Developments in Dairy Chemistry*, (ed. P.F. Fox), Applied Science Publishers, London, Vol. 3, pp. 281–336.

Reiter, B. (1985b) Interaction between immunoglobulins and innate factors such as lysozyme, lactoferrin, lactoperoxidase, in *Composition and Physiological Properties of Human Milk*, (ed. J. Schaub), Elsevier, Amsterdam, pp. 271–284.

Renner, E., Schaafsma, G. and Scott, K.J. (1989) Micronutrients in milk in, *Micronutrients in Milk and Milk-based Food Products*, (ed. E. Renner), Elsevier, New York, pp. 1–70.

Reynolds, M., Linzell, J.L. and Rasmussen, F. (1968) Comparison of four methods for measuring mammary blood flow in conscious goats. *American Journal of Physiology*, **214**, 1415–1424.

Ribadeau-Dumas, B., Brignon, G., Grosclaude, F. and Mercier, J.C. (1972) Structure primaire de la caséine β bovine. Séquence complete. *European Journal of Biochemistry*, **25**, 505–514.

Richardson, B.C. and Mercier, J.C. (1979) The primary structure of the ovine β-caseins. *European Journal of Biochemistry*, **99**, 285–297.

Robinson, A.M., Girard, J.R. and Williamson, D.H. (1978) Evidence for a role of insulin in the regulation of lipogenesis in lactating rat mammary gland. *Biochemical Journal*, **176**, 343–346.

Robinson, D.S. (1963) Changes in the lipolytic activity of the guinea pig mammary gland at parturition. *Lipid Research*, **4**, 21–23.

Robinson, D.S., Jefferies, G.H. and Poole, J.C.F. (1955) Further studies on the interaction of chyle and plasma in the rat. *Quarterly Journal of Experimental Physiology*, **40**, 297–308.

Rogan, W.J. (1986) Epidemiology of environmental chemical contaminants in breast milk, in *Human Lactation 2: Maternal and Environmental Factors*, (eds M. Hamosh and A.S. Goldman), Plenum Press, New York, pp. 437–446.

Rogers, S., Wells, R. and Rechsteiner, M. (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science*, **234**, 364–368.

Rook, A.J.F. and Campling, R.C. (1965) Effect of stage and number of lactation on the yield and composition of cow's milk. *Journal of Dairy Research*, **32**, 45–55.

Rosen, J.M. (1987) Milk protein gene structure and expression, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 301–322.

Rosen, J.M., Matusik, R.J., Richards, D.A., Gupta, P. and Rodgers, J.R. (1980) Multihormonal regulation of casein gene expression at the transcriptional and posttranscriptional level in the mammary gland. *Recent Progress in Hormone Research*, **36**, 157–194.

Roth, J. and Berger, E.G. (1982) Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. *Journal of Cell Biology*, **93**, 223–229.

Roy, J.H.B. and Smith, T. (1987) Rearing calves and heifers, in *Dairy Cattle Production*, (ed. H.O.

Gravert), World Animal Science C3, Elsevier, Amsterdam, pp. 251–268.

Russo, J. and Russo, I.H. (1987) Development of the human mammary gland, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 67–93.

Saake, R.G. and Heald, C.W. (1974) Cytological aspects of milk formation and secretion, in *Lactation: a Comprehensive Treatise*, Vol. 2, (eds B.L. Larson and V.R. Smith), Academic Press, New York, pp. 147–189.

Saltiel, A.R. and Cuatrecasas, P. (1986) Insulin stimulates the generation from hepatic plasma membranes of modulators derived from an inositol glycolipid. *Proceedings of the National Academy of Sciences, USA*, **83**, 5793–5797.

Schade, A.L. and Reinhart, R.W. (1970) Galactothermin, a reversibly heat-precipitable protein of human milk at neutral pH. *Biochemical Journal*, **118**, 181–186.

Schaub, J. (1985) *Composition and Physiological Properties of Human Milk*, Elsevier, Amsterdam.

Schmidt, D.G. (1980) Colloidal aspects of casein. *Netherlands Milk and Dairy Journal*, **34**, 42–64.

Schmidt, D.G. (1982) Association caseins and casein micelle structure, in *Developments in Dairy Chemistry*, Vol. 1, (ed. P.F. Fox), Applied Science Publishers, New York, pp. 61–86.

Schmidt, G.H. (1971) *Biology of Lactation*, W.H. Freeman, San Francisco.

Schoefl, G.I. and French, J.E. (1968) Vascular permeability to particulate fat: morphological observations on vessels of lactating mammary gland and of lung. *Proceedings of the Royal Society of London Series* B 169, 153–165.

Scow, R.O., Hamosh, M., Blanchette-Mackie, E.J. and Evans, A.J. (1972) Uptake of blood triglyceride by various tissues. *Lipids*, **7**, 497–505.

Shing, Y.W., Dai, S. and Klagsbrun, M. (1985) Detection of three growth factors, HMGF 1, HMGF 11 and HMGF 111 in human milk, in *Human Lactation: Milk Components and Methodologies*, (eds R.J. Jensen and M.C. Neville), Plenum Press, New York, pp. 201–208.

Short, R.V. (1976) Lactation – the central control of reproduction, in *Breastfeeding and the Mother*, Ciba Foundation Symposium 45, Elsevier, Amsterdam, p. 73–86.

Silverman, M. (1991) Structure and function of hexose transporters. *Annual Reviews of Biochemistry*, **60**, 757–794.

Slattery, C.W. (1978) Variation in the glycosylation pattern of bovine κ-casein with micelle size and its relationship to a micelle model. *Biochemistry*, **17**, 1100–1104.

Slattery, C.W. (1979) A phosphate-induced submicelle equilibrium in reconstituted casein micelle systems. *Journal of Dairy Research*, **46**, 253–258.

Slattery, C.W. and Evard, R. (1973) A model for the formation and structure of casein micelles from subunits of variable composition. *Biochimica et Biophysica Acta*, **317**, 529–538.

Slot, J.W. and Geuze, H.J. (1983) Immunoelectron microscopic exploration of the Golgi complex. *The Journal of Histochemistry and Cytochemistry*, **31**, 1049–1056.

Smith, A.J. (ed.) (1985) *Milk production in developing countries*. Centre for Tropical Veterinary Medicine, Edinburgh.

Smith, S. and Abraham, S. (1975) The composition and biosynthesis of milk fat. *Advances in Lipid Research*, **13**, 195–239.

Smith, S. and Ryan, P. (1979) Asynchronous appearance of two enzymes concerned with medium chain fatty acid synthesis in developing rat mammary gland. *Journal of Biological Chemistry*, **254**, 8932–8936.

Smith, S.G., Lewis, M., Aschaffenburg, R., Fenna, R.E., Wilson, I.A., Sundaralingham, M., Stuart, D.I. and Phillips, D.C. (1987) Crystallographic analysis of the three-dimensional structure of baboon α-lactalbumin at low resolution. *Biochemical Journal*, **242**, 353–360.

Solari, R. and Kraehenbuhl, J.P. (1987) Receptor-mediated transepithelial transport of polymeric immunoglobulins, in *The Mammary Gland: Development, Regulation and Function*, (eds M.C. Neville and C.W. Daniel), Plenum Press, New York, pp. 269–298.

Soulier, S., Sarfati, R.S. and Szabó, L. (1980) Structure of the asialyl oligosaccharide chains of κ-casein isolated from ovine colostrum. *European Journal of Biochemistry*, **108**, 465–472.

Spik, G., Strecker, G., Fornet, B., Bouquelet, S., Montreuil, J., Dorland, L., Van Halbeek, H. and Vliengenthart, F.G. (1982) Primary structure of the glycans from human lactotransferrin. *European Journal of Biochemistry*, **121**, 413–419.

Srivastava, D.K. and Bernhard, S.A. (1986) Metabolite transfer via enzyme–enzyme complexes. *Science*, **234**, 1081–1086.

Stein, O. and Stein, Y. (1967) Lipid synthesis, intracellular transport and secretion. II. electron microscopic radioautographic study of mouse

lactating mammary gland. *Journal of Cell Biology*, **34**, 251–263.

Strong, C.R. and Dils, R.R. (1972) Fatty acid and biosynthesis in rabbit mammary gland during pregnancy and early lactation. *Biochemical Journal*, **128**, 1303–1309.

Stuart, D.I., Acharya, K.R., Walker, N.P.C., Smith, S.J., Lewis, M. and Phillips, D.C. (1986) α-lactalbumin possesses a novel calcium binding loop. *Nature (London)*, **324**, 84–87.

Takeuchi, M., Tsuda, E., Yoshikawa, M., Sasaki, R. and Chiba, H. (1984) Post-translational modification of κ-casein in the Golgi apparatus of mid-lactating mammary glands from rat and cow. *Agricultural and Biological Chemistry*, **48**, 2789–2797.

Talbot, B. and Waugh, D.F. (1970) Micelle-forming characteristics of monomeric and covalent polymeric κ-caseins. *Biochemistry*, **9**, 2807–2813.

Teschemacher, H. (1987) β-casomorphins: do they have physiological significance?, in *Human Lactation 3: the Effects of Human Milk on the Recipient Infant*, (eds A.S. Goldman, S.A. Atkinson and L.A. Hanson), Plenum Press, New York, pp. 213–225.

Teyssot, B. and Houdebine, L.M. (1980) Role of prolactin in the transcription of β-casein and 28-s ribosomal genes in the rabbit mammary gland. *European Journal of Biochemistry*, **110**, 263–272.

Teyssot, B. and Houdebine, L.M. (1981) Role of progesterone and glucocorticoids in the transcription of the β-casein and 28-s ribosomal genes in the rabbit mammary gland. *European Journal of Biochemistry*, **114**, 597–608.

Threadgold, L.C. and Kuhn, N.J. (1979) Glucose-6-phosophate hydrolysis by lactating rat in mammary gland. *International Journal of Biochemistry*, **10**, 683–685.

Threadgold, L.C. and Kuhn, N.J. (1984) Monosaccharide transport in the mammary gland of the intact lactating rat. *Biochemical Journal*, **218**, 213–219.

Threadgold, L.C., Coore, H.G. and Kuhn, N.J. (1982) Monosaccharide transport into lactating rat mammary acini. *Biochemical Journal*, **204**, 493–504.

Topper, Y.J. (1970) Multiple hormone interactions in the development of mammary gland *in vitro*. *Recent Progress in Hormone Research*, **26**, 287–308.

Trayer, I.P. and Hill, R.L. (1971) The purification and properties of the A protein of lactose synthetase. *Journal of Biological Chemistry*, **246**, 6666–6675.

Turkington, R.W. (1971) Hormonal control of lactose synthetase in developing mammary gland, in *Hormones in Development*, (eds M. Hamburgh and E.J.W. Barrington), Appleton-Century-Crofts, New York, pp. 49–59.

Turkington, R.W. and Hill, R.L. (1968) Lactose synthetase: progesterone inhibition of the induction of α-lactalbumin. *Science*, **163**, 1458–1460.

Vab Halbeek, H., Dorland, L., Vliengenthart, J.F.G., Fiat, A.M. and Jollès, P. (1980) MHz ^{1}H-NMR study of three oligosaccharides isolated from cow κ-casein. *Biochimica et Biophysica Acta*, **623**, 295–300.

Van Valen, L. (1960) Therapsids as mammals. *Evolution*, **14**, 304–313.

Vernon, R.G. and Flint, D.S. (1984) Adipose tissue: metabolic adaption during lactation. *Symposia of the Zoological Society of London*, **51**, 119–145.

Visser, S., Jenness, R. and Mullin, R.J. (1982) Isolation and characterization of β- and α-caseins from horse milk. *Biochemical Journal*, **203**, 131–139.

Vondehaar, B.K. and Nakhasi, H.L. (1986) Bifunctional activity of epidermal growth factor on α- and κ-casein gene expression in rodent mammary glands *in vitro*. *Endocrinology*, **119**, 1178–1184.

Vorherr, H. (1978) Human lactation and breast feeding, in *Lactation: a Comprehensive Treatise*, Vol. 4, (ed. B.L. Larson), Academic Press, New York, pp. 181–280.

Vreeman, H.J., Visser, S., Slangen, C.J. and Van Riel, J.A.M. (1986) Characterization of bovine κ-casein fractions and the kinetics of chymosin-induced macropeptide release from carbohydrate-free and carbohydrate-containing fractions determined by high-performance gel-permeation chromatography. *Biochemical Journal*, **240**, 87–97.

Wang, D.Y., Hallowes, R.C., Bealing, J., Strong, C. and Dils, R.R. (1971) Effect of prolactin and growth hormone on fatty acid synthesis in mouse mammary gland explants in organ culture. *Journal of Endocrinology*, **51**, xxx–xxxi.

Watkins, W.M. and Hassid, W.Z. (1962) The synthesis of lactose by particulate enzyme preparations from guinea pig and bovine mammary glands. *Journal of Biological Chemistry*, **237**, 1432–1440.

West, C.E., Bickerstaffe, R., Annison, E.F. and Linzell, J.L. (1972) Studies on the mode of up-

take of blood triglycerides by the mammary gland of the lactating goat. *Biochemical Journal*, **126**, 477–490.

West, D.W. and Clegg, R.A. (1983) Casein kinase activity in rat mammary gland Golgi vesicles. *European Journal of Biochemistry*, **137**, 215–220.

West, D.W. and Clegg, R.A. (1984) Casein kinase activity in rat mammary gland Golgi vesicles. Demonstration of latency and requirement for a transmembrane ATP carrier. *Biochemical Journal*, **219**, 181–187.

White, M.D., Kuhn, N.J. and Ward, S. (1980) Permeability of lactating rat mammary gland Golgi membranes to monosaccharides. *Biochemical Journal*, **190**, 621–624.

White, M.D., Kuhn, N.J. and Ward, S. (1981) Mannitol and glucose movement across the Golgi membrane of lactating-rat mammary gland. *Biochemical Journal*, **194**, 173–177.

White, M.D., Ward, S. and Kuhn, N.J. (1982) Biosynthesis of galactinol by lactose synthetase. *International Journal of Biochemistry*, **14**, 449–451.

White, M.D., Ward, S. and Kuhn, N.J. (1984) Pore properties of the Golgi membrane from lactating-rat mammary gland. Effects of pH and temperature and reconstitution into phospholipid vesicles. *Biochemical Journal*, **217**, 297–301.

Wickler, W. (1969) *The Sexual Code*, Weidenfeld & Nicolson, London, pp. 184–185.

Wilde, C.J. and Kuhn, N.J. (1979) Lactose synthesis in the rat, and the effects of litter size and malnutrition. *Biochemical Journal*, **182**, 287–294.

Wilde, C.J. and Peaker, M. (1990) Autocrine control in milk secretion. *Journal of Agricultural Research*, **114**, 235–238.

Wilde, C.J., Calvert, D.T., Daly, A. and Peaker, M. (1987) The effect of goat milk fractions on synthesis of milk constituents by rabbit mammary explants and on milk yield *in vivo*. *Biochemical Journal*, **242**, 285–288.

Williamson, D.H. (1990) The lactating mammary gland of the rat and the starved–refed transition: a model system for the study of the temporal regulation of substrate utilization. *Biochemical Society Transactions*, **18**, 853–856.

Williamson, D.H., Munday, M.R., Jones, R.G., Roberts, A.F.C. and Ramsey, A.J. (1983) Short-term dietary regulation of lipogenesis in the lactating mammary gland of the rat. *Advances in Emzyme Regulation*, **21**, 135–145.

Wilson, E.O. (1975) *Sociobiology: The New Synthesis*, Harvard University Press, Cambridge, Massachusetts, pp. 341–344.

Wong, R.S.C. and Bennick, A. (1980) The primary structure of a salivary calcium-binding proline-rich phosphoprotein (Protein C), a possible precursor of a related salivary protein A. *Journal of Biological Chemistry*, **255**, 5943–5948.

Wood, H.G., Peeters, G.J., Verbeke, R., Lauryssens, M. and Jacobson, B. (1965) Estimation of the pentose cycle in the perfused cow's udder. *Biochemical Journal*, **96**, 607–615.

Wooding, F.B.P. (1977) Comparative mammary fine structure. *Symposia of the Zoological Society of London*, **41**, 1–41.

Wooding, F.B.P. and Morgan, G. (1978) Calcium localization in lactating rabbit mammary secretory cells. *Journal of Ultrastructural Research*, **63**, 323–333.

World Health Organization (1981) *Contemporary Patterns of Breast Feeding*. WHO, Geneva.

World Health Organization (1985) *The Quantity and Quality of Breast Milk*. WHO, Geneva.

Yip, M.C.M. and Knox, W.E. (1972) Function of arginase in lactating mammary gland. *Biochemical Journal*, **127**, 893–899.

Young, T. (1761) De Lacte, in *Thesaurus Dissertationem*, Vol. 2, (ed E. Sandifort), Rotterdam.

Yudilevich, D.L., Eaton, B.M., Short, A.H. and Leichtweiss, H.P. (1979) Glucose carriers at the maternal and fetal sides of the trophoblast in guinea pig placenta. *American Journal of Physiology*, **237**, C205–C212.

Yu–Lee, L. and Rosen, J.M. (1983) The rat casein multigene family. 1. Fine structure of the γ-casein gene. *Journal of Biological Chemistry*, **258**, 10794–10804.

Yu–Lee, L., Richter-Mann, L., Couch, C.H., Stewart, A.F., Mackinlay, A.G. and Rosen, J.M. (1986) Evolution of the casein multigene family: conserved sequences in the 5′ flanking and exon regions. *Nucleic Acids Research*, **14**, 1883–1902.

Zaczek, M. and Keenan, T.W. (1990) Morphological evidence for an endoplasmic reticulum origin of milk lipid globules obtained using lipid-selective staining procedures. *Protoplasma*, **159**, 179–182.

Zinder, O., Hamos, M., Fleck, T.R.C. and Scow, R.O. (1974) Effect of prolactin on lipoprotein lipase in mammary gland and adipose tissue of rats. *American Journal of Physiology*, **226**, 744–748.

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